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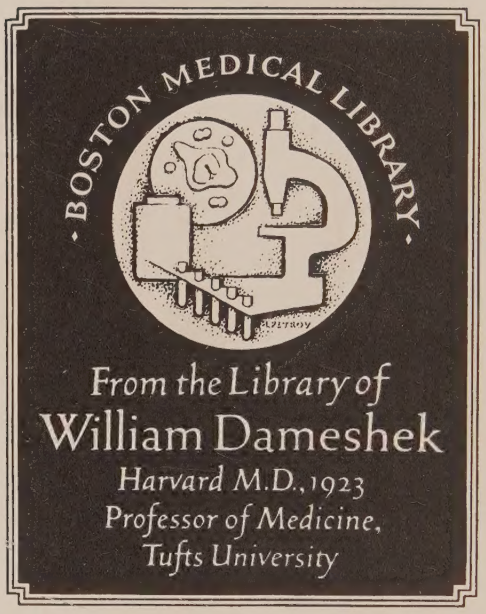
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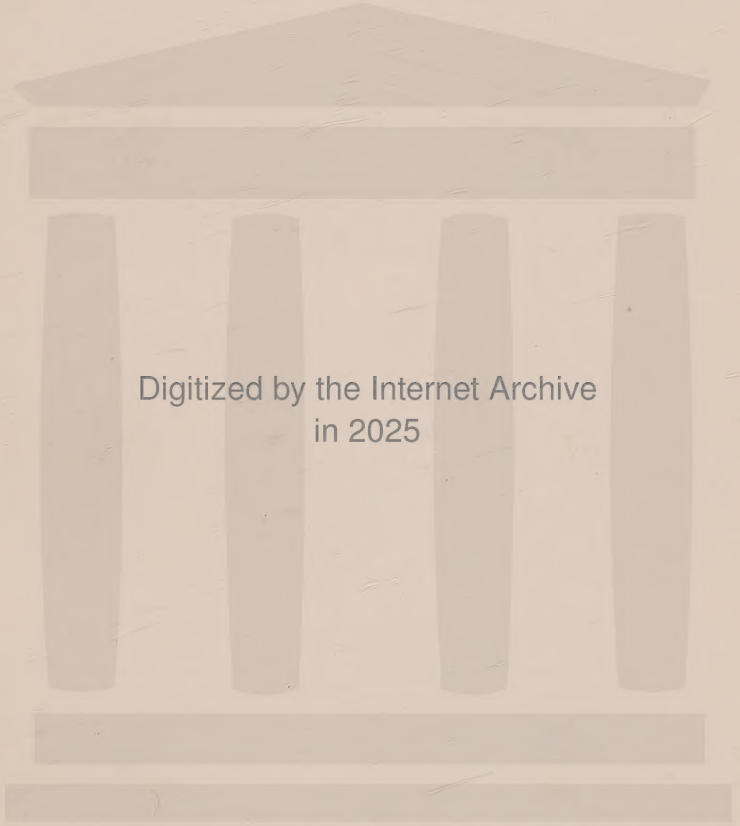
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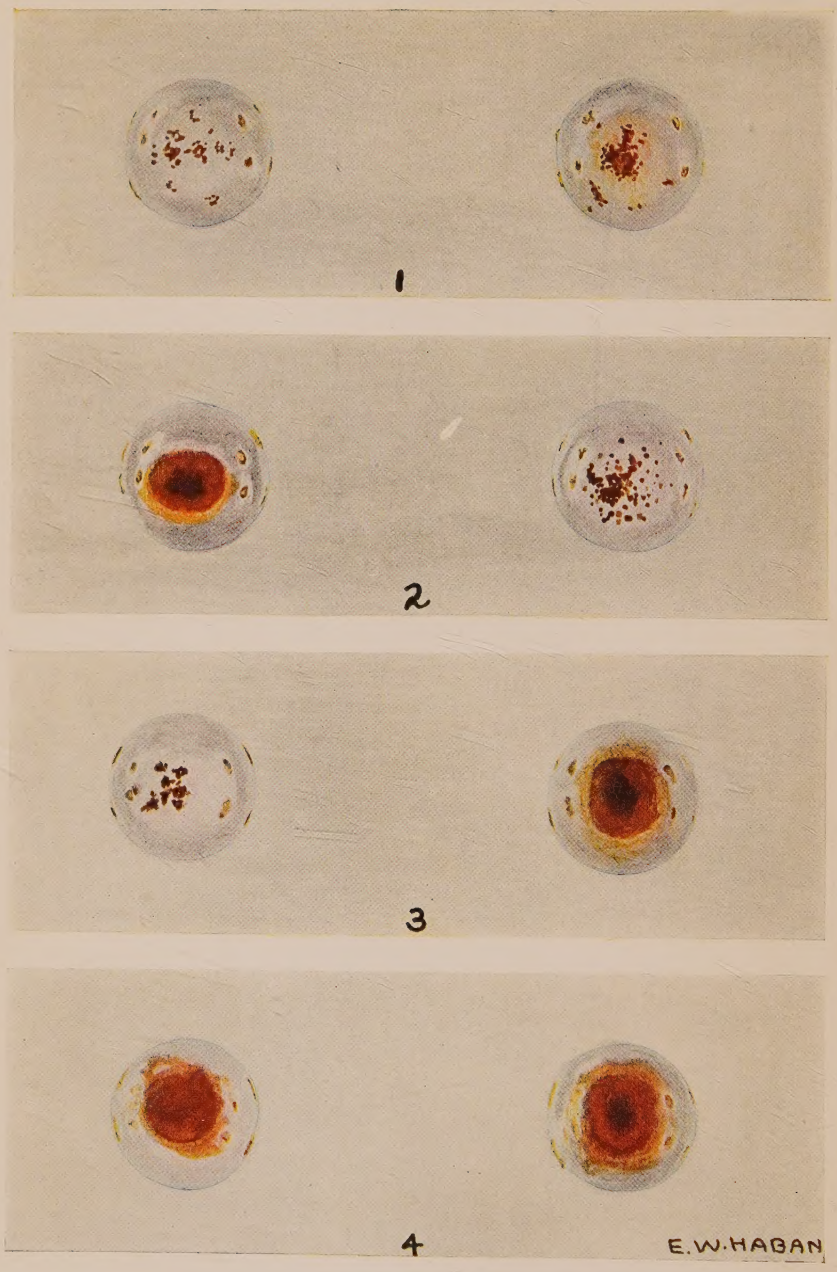

THE FOUR BLOOD GROUPS

Serum of group *A* on the left, serum of group *B* on the right. Unknown cells mixed with both serums on each slide. Slide 1, group *AB;* slide 2, group *A;* slide 3, group *B;* slide 4, group *O*.

BLOOD GROUPING IN RELATION TO CLINICAL AND LEGAL MEDICINE

BY

LAURENCE H. SNYDER, Sc.D.

Associate Professor of Zoölogy North Carolina State College, Raleigh; Committee on Blood Grouping, National Research Council

BALTIMORE
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To

LUDWIG HEKTOEN

PREFACE

With the dawn of the twentieth century there was born a problem which, at first unnoticed and unencouraged, has nevertheless kept pace with the years in its growth, and bids fair to take a prominent place in the annals of human biology. The blood group problem, originally of purely theoretical interest, first demonstrated its practical importance to the question of blood transfusion. Later it invaded the field of legal medicine, providing an approach to the solution of the problems of disputed parentage. The theoretical interest in the blood groups rapidly widened to include the genetical and anthropological aspects of the phenomena. And finally, at the present time, interest, both theoretical and practical, is centering around the applications of blood grouping to clinical medicine other than transfusion, that is, to the genetic and ontogenetic relationships of blood groups to various morphologic, pathologic, and physiologic conditions. It is fitting, then, that these various applications and trends of interest be brought together under one cover, in order that students of medicine, genetics, and anthropology may meet on common ground as a starting point for such further investigations or lines of thought as they may care to undertake.

The literature on the various phases of the subject covers such a wide range of publications and languages that it has been difficult to cite directly in the body of the book each source of information. I have used freely material from the articles of many investigators, and have attempted to give credit for such major ideas as have been incorporated into the present work. A rather complete bibliography is appended at the end of the book.

My viewpoint in treating the subject has been mainly that of the geneticist and physiologist. My own experimental work has involved the questions of the inheritance of the groups, their racial distribution, their appearance in animals, and the relation of the human blood groups to various pathological and physiological conditions. Much of this last has been carried out in various hospitals, mainly in North Carolina. In this connection I have acquired some experience in performing transfusions, using various technics involving both whole and citrated blood. As the most important practical application of blood grouping, transfusion has been thoroughly discussed in the following chapters in addition to the more theoretical applications.

To the various authors and publishers who have kindly allowed the use of their illustrations my thanks are due. To the many physicians of North Carolina who have aided me in obtaining data, I am extremely indebted. And to the committee on scientific research of the American Medical Association, whose generous support permitted many of the investigations which I have personally carried on, my gratitude is forthcoming. To Dr. Ludwig Hektoen I am especially grateful, and to him this book is affectionately dedicated.

LAURENCE H. SNYDER

Raleigh, June 1929

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CHAPTER I

The Discovery of the Blood Groups

"Vor einige Zeit habe ich beobachtet dass öfters Blutserum von normalen Menschen rothe Blutkörperchen anderer gesunder Individuen zu verklumpen im Stande ist. . . . . Es ist also möglicher Weise die Reaction in manchen Fällen zur Identificirung, oder besser gesagt, zur Erkennung der Nichtidentität von Blutproben, etwa zu Forensichen Zwecken geeignet. . . . Endlich sei noch erwähnt, dass die angeführten Beobachtungen die wechselden Folgen therapeutischer Menschenbluttransfusionen zu erklären gestatten."—Landsteiner, *Ueber Agglutinationserscheinungen normalen menschlichen Blutes*, 1901.

The discovery of "blood groups" within the human race, while coming as an unexpected and surprising phenomenon, took place in a more or less prepared atmosphere. Studies in immunity had been for some time progressing rapidly under the leadership of Ehrlich and his co-workers, in Germany. By the close of the nineteenth century hemolysis had been especially investigated, and iso-, auto-, and hetero-hemolysis differentiated. Thus it was realized that individual blood differences could occur within a species. Agglutination studies were likewise in progress in various laboratories, and Bordet in Paris had demonstrated the possibility of removing agglutinins by absorption.

In 1899 Shattock, in England, presented the results of observations on human blood in certain pathological conditions. He noted a tendency for the red cells to settle and agglomerate instead of remaining as separate entities. This tendency, often quoted as the first recorded case of iso-agglutination, was in reality probably only a case of pseudo-agglutination, as will be pointed out later.

During the years 1900 and 1901, Karl Landsteiner, an Austrian investigator at present connected with the Rockefeller Institute in New York, observed that when the serum and cells of normal human beings were mixed, the erythrocytes of one person were frequently caused to adhere in groups or clumps by the serum of another person. This reaction (agglutination) was known to occur when erythrocytes were mixed with serum from another species, i.e., "foreign" or "heterologous" serums; but it was an entirely new discovery that it could occur within a species. Following Ehrlich's nomenclature for hemolysis, the reaction was called "iso-agglutination." In a study of the bloods of twenty-two persons, Landsteiner found that this

reaction did not take place at random, but that there existed certain definite relationships between the bloods of various normal human beings. The bloods of these twenty-two persons could be placed in three groups.

The serum of one group agglutinated the erythrocytes of the other two groups, but not its own erythrocytes; its own erythrocytes moreover were not agglutinated by any serums. This group he called *C*. The serum of another group (*A*) agglutinated the corpuscles of the third group (*B*), but not those of *A* or *C*; the corpuscles of this group (*A*) were agglutinated by the serums of *B* and *C*, but not of *A*. The serum of the third group (*B*) agglutinated the corpuscles of the second group (*A*), but not those of *B* or *C*; its corpuscles were agglutinated by the serums of *A* and *C*, but not by that of *B*.

From these results, Landsteiner postulated the presence in the serum of two specific iso-agglutinins, and in the erythrocytes two corresponding iso-agglutinogens, independent of any causal relationship to disease. The agglutinins could appear together in a blood, or either one could appear singly. Correspondingly, the agglutinogens could both be absent from a blood, or could appear singly. Landsteiner found no cases where the agglutinins were both absent and the agglutinogens both present. Such cases were found and described the following year (1902), however, by von Decastello and Sturli. Sturli had coöperated in Landsteiner's earlier work, and pursued the investigations with Landsteiner's approval. The cases described by von Decastello and Sturli formed a fourth group, and were found in a relatively infrequent proportion of the cases studied. Only the fact that relatively large numbers were used made it possible for this group to be detected at all. Four cases were observed in the 155 persons studied. To this day all peoples investigated have shown the same small percentage of this group.

Landsteiner's assumption of two iso-agglutinins and two iso-agglutinogens in human blood was further elaborated by the assumption that these elements were reciprocal: that is, that the presence of an agglutinogen in a blood precluded the presence of the corresponding agglutinin, and that the absence of an agglutinogen implied the presence of the corresponding agglutinin. The presence in the serum of true iso-agglutinins was confirmed by von Decastello and Sturli by absorption experiments.

Hektoen, in 1907, confirmed Landsteiner's three groups and also the additional group found by von Decastello and Sturli. Jansky, in 1907, made the first definite classification of the four groups, numbering them I, II, III, and IV. The Jansky classification is as follows:

Group I. Serum agglutinates the corpuscles of groups II, III and IV. Corpuscles not agglutinated by any serum.

Group II. Serum agglutinates the corpuscles of groups III and IV, but not those of I and II. Corpuscles agglutinated by serums of groups I and III, but not by those of groups II and IV.

Group III. Serum agglutinates the corpuscles of groups II and IV, but not those of I and III. Corpuscles agglutinated by serums of groups I and II, but not by those of groups III and IV.

Group IV. Serum does not agglutinate the corpuscles of any group. Corpuscles agglutinated by serums of groups I, II, and III.

Landsteiner, in 1909, gave a complete description of the four groups. Moss, in 1909 and 1910 in this country, devised a classification similar to Jansky's, in which groups I and IV of Jansky's were reversed, II and III remaining the same, judging by their frequency in the population.

Jansky's article being in an obscure and inaccessible publication, Moss's work became the standard for group classification in America in parts of Europe. Jansky's grouping was followed enough, however, to result in a dangerous confusion arising from the use of two classifications. In recognition of this confusion, a committee in America representing the American Association of Immunologists, the Society of American Bacteriologists, and the Association of Pathologists and Bacteriologists, in 1921 recommended that the Jansky classification be universally adopted on the basis of priority. At that time about 90 per cent of the hospitals which were doing blood grouping were using the Moss classification. The recommendation has not been followed, however, and a recent survey of the situation by Kennedy (1929) indicates that 78 per cent of those hospitals doing blood grouping are still using the Moss system.

To try to obviate the confusion still existing from a reversal of groups I and IV in the two systems, a new system of nomenclature, based on the agglutinophyllic capacity of the cells, has been suggested. In this system, Jansky's group IV is known as *AB*, having the two agglutinogens A and B. Group III, containing agglutinogen B, is known as group *B*, group II as group *A*, and group I, containing neither agglutinogen, as group *O*. If this system is universally adopted, no confusion can result. The correlation of the various systems is shown in table 1.

The system of designating the groups by letters has been used in nearly all of the important recent publications. A warning, however, is sounded by Kennedy in his recent survey, in which he confidently predicts that continued agitation for the new terminology will only result in the use of three classifications, a condition to be avoided if possible. The new classification, he goes on to say, further commits those using it to a definite

agglutinogen content of each of the groups. This, as will be seen in chapter III, is debatable. A further point of disadvantage is that certain authors in the past have used capital letters to designate the agglutinins instead of the agglutinogens, thus adding to the confusion.

The new terminology, however, is in my opinion so far superior to the confusion caused by the similarity of the other two, that it will be used throughout this work. The scheme of this classification is given in table 2.

Like many other discoveries destined to become milestones in human progress, Landsteiner's findings were practically unnoticed for years. Occasional writers called attention to their importance, but no general

TABLE 1

CORRELATION OF THE VARIOUS CLASSIFICATIONS OF BLOOD GROUPS

LANDSTEINER'S ORIGINAL CLASSIFICATION	JANSKY	MOSS	PROPOSED NEW DESIGNATIONS
C	I	IV	*O*
A	II	II	*A*
B	III	III	*B*
—	IV	I	*AB*

TABLE 2

SCHEME OF THE PROPOSED NEW CLASSIFICATION OF BLOOD GROUPS

GROUP	EFFECT OF SERUM	CAPACITY OF CELLS
O	Agglutinates cells of *A*, *B*, *AB*	Not agglutinated by any serum
A	Agglutinates cells of *B* and *AB*	Agglutinated by serums of *O* and *B*
B	Agglutinates cells of *A* and *AB*	Agglutinated by serums of *O* and *A*
AB	Does not agglutinate any cells	Agglutinated by serums of *O*, *A*, *B*

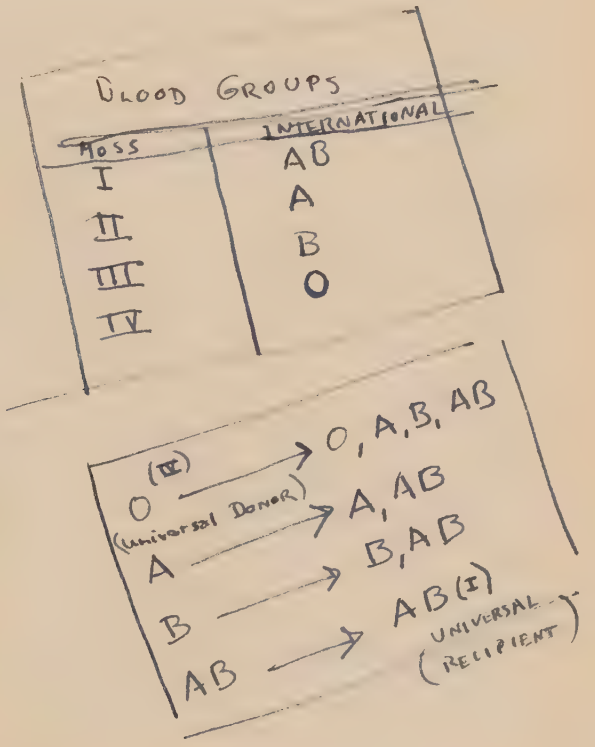
consideration was given them, and this in spite of the fact that Landsteiner had specifically pointed out two fundamental practical applications of his discoveries. These were the importance of the blood groups in explaining the untoward reactions occurring after transfusions, and the possible applications of the groups to medico-legal problems.

Until the World War, however, so little attention was paid to this work that during the early part of the war many transfusions were fatal because of the agglutination reaction occurring in the patient's blood vessels due to the use of incompatible donors. The necessity for large numbers of emergency transfusions brought the problem sharply to the fore, however, and recourse was had to the long-obscured knowledge of isohemagglutina-

tion. The use of donors whose compatibility was determined by blood grouping resulted in successful transfusions, and the blood groups emerged from their obscure position to one of foremost importance to medical science. Transfusion thus became the first real practical application of blood grouping.

The experiments of H. and L. Hirszfeld, carried out on the battlefront, likewise opened new possibilities for the blood group question. These experiments in the racial distribution of the groups will be discussed in chapter XIV, but their importance in initiating research along these lines may be indicated here. In the years following the war, investigators in all parts of the world have attacked all sides of the blood group problem with such vigor that there has grown up a large body of knowledge on the subject, and the practical importance of blood grouping to clinical and legal medicine, as well as its theoretical importance in genetics, eugenics, anthropology, physiology, and immunology, are well known.

After a discussion of the iso-agglutination reaction itself, then, these practical and theoretical applications will be taken up in detail.



CHAPTER II

Isohemagglutination

"Agglutination, like other immunity reactions, is a manifestation of broad biologic laws."—Kolmer, *Infection, Immunity, and Biologic Therapy,** 1924.

The phenomenon of agglutination is an immunological phenomenon. It becomes of interest to the transfusion problem only as it affects the integrity of a donor's erythrocytes in the blood stream of the recipient. To other problems connected with the blood groups in clinical and legal medicine its application lies in the hereditary nature of the groups themselves. From the immunological standpoint, however, certain fundamental matters pertaining to agglutination underlie a proper understanding of this phenomenon as it occurs in red blood cells, and it will therefore be briefly discussed.

Certain substances, chiefly soluble proteins, when introduced into the body of an animal cause the animal to produce definite antibodies which may combine specifically with the introduced substance. The introduced substance is known as an antigen. Antigens are apparently always colloidal in nature.

The antibodies formed by an antigen are generally specific for the antigen. Union between an antigen and its specific antibody always takes place when they are brought together in solution. The union is a physicochemical reaction, and may not destroy the identity of either component. Antibodies are frequently found in normal serums, and such are known as normal antibodies, as contrasted to those stimulated by immunization, which are called immune antibodies.

The antigen-antibody reaction may have various manifestations. A poisonous antigen may be neutralized by union with the antibody (toxin-antitoxin reaction). A soluble antigen may form a precipitate upon union with the antibody (precipitation reaction). A cellular antigen may be dissolved by union with the antibody (lytic reactions such as cytolysis or hemolysis). Or the cells of a particulate antigen such as bacteria or red blood corpuscles may upon union with the antibody adhere and form clumps, which settle, leaving a clear supernatant fluid (agglutination reaction).

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The agglutination of red blood cells is known as hemagglutination, and the agglutination of red blood cells by the serum of the same species is known as isohemagglutination. The antigen in the red cells is known as an agglutinogen, and the antibody in the serum as an agglutinin. The agglutinogens concerned in the human blood groups are thus isohemagglutinogens, and the corresponding agglutinins are isohemagglutinins.

In addition to serum hemagglutinins, other agents may cause the agglutination of erythrocytes. Among them are certain plant poisons (abrin, crotin, ricin, etc.), certain bacterial substances (staphylococcus, B. typhosus, etc.), certain inorganic colloids, snake venom, milk, colostrum, amniotic fluid, and others. Likewise agglutinogens similar to, if not identical with the hemagglutinogens, have been found in various substances. Among these may be mentioned human spermatozoa, seminal fluid, vaginal secretions, saliva, and other body fluids and body organs. Absorption, precipitation, and immunization methods have been used in demonstrating these. Our chief interest lies, however, in the specific isohemagglutinins in human serums, and in the specific agglutinogens in human red blood cells, since these form the basis for the human blood groups.

Agglutination has long been considered as a special case of the flocculation of a colloidal suspension. As our knowledge of the mechanism of such flocculations has advanced, our understanding of the mechanism of agglutination has increased, although conflicting opinions have not as yet been reconciled. It is generally conceded that cells in suspension are kept apart by mutual repulsion due to their like charges. An opposing force, an attraction, must also exist. This force may be surface tension. If the forces tending to draw the cells together are greater than those tending to keep them apart, agglutination will occur. The agglutinin-agglutinogen reaction results in overcoming the forces of repulsion, perhaps by changing the difference of potential between the cells and the surrounding medium, perhaps by some other method. The high specificity of the reaction adds to the complexity of the problem.

Schroder (1926) observed that isohemagglutination is always accompanied by a decrease in the electrical charge on the erythrocytes. She observed the smallest absolute charge in erythrocytes of groups with strong agglutination; the largest, in erythrocytes of all groups mixed with serum of group *AB*, and for erythrocytes of group *O:* combinations with complete absence of agglutination.

Whatever the mechanism of agglutination, the fact remains that there are in human serums certain isohemagglutinins, and in human erythrocytes certain agglutinogens, variously arranged according to definite laws. Landsteiner, in order to explain his discoveries, postulated two iso-agglutinins

in the serum, and two corresponding agglutinogens in the red cells. The further assumption of the essential reciprocity of these elements has already been mentioned. On this basis the four blood groups are built as outlined in the first chapter.

The discovery that a blood could contain agglutinins against cells of its own species was rather unexpected, as it had been taken for granted that a substance found naturally in the tissues of a species could not act as an antigen for that species. Here, however, is a case where the specificity is restricted to individual circulations rather than to a species. This led to Landsteiner's law of reciprocity, mentioned above; a law which has been generally accepted. Recently, however, the hypothesis of multiple allelomorphism as a basis for the heredity of the groups has presented a new possibility as to the nature of the serums. The multiple allelomorph hypothesis will be discussed in chapter X. Suffice it to say here that on this basis the serums of all groups are considered identical, containing agglutinins a and b, and a third hypothetical agglutinin o. The various bloods differ in their agglutinogens, these differences being determined by heredity. An equilibrium process of reciprocal binding prevents autoagglutination. Thus the essential reciprocity of agglutinin and agglutinogen in Landsteiner's scheme is considered merely a seeming one.

This view, elaborated by Bernstein, may have merit in the light of the overwhelming confirmation of the multiple allelomorph hypothesis of heredity. It may be summarized as follows. In the serum of each of the six genetical types there will be present the agglutinins a, b, and o, with the peculiarity that in each case the agglutinin corresponding to the agglutinogen present will be rendered inactive by reciprocal binding. This is indicated schematically by parentheses. Morever, the agglutinin o and its corresponding agglutinogen O take a special position, since due to the hereditary recessivity of the gene O on the one hand, and from as yet unknown causes on the other hand, a direct action of these hypothetical factors is not yet proven. This is indicated by a superimposed "°." From the foregoing is constructed the following scheme:

Group.....	*O*	*A*		*B*		*AB*
Agglutinogens.....	ÖÖ	AÖ	AA	BÖ	BB	AB
Agglutinins.....	ab(ö)	(a)b(ö)	(a)bö	a(b) (ö)	a(b)ö	(a) (b)ö

Landsteiner's scheme may be regained from this by the omission of the characters in parentheses and the recessive non-acting "o." In support of this view Schiff (1927) found that after mixing beef serum with erythrocytes of group *AB*, the serum still agglutinated electively human corpuscles of group *O*. This is evidence for a true receptor in group *O*, not merely the

absence of agglutinogens A and B. Corpuscles of groups *A* and *B* were not agglutinated by this serum, although it might be expected that the erythrocytes of heterozygous individuals of groups *A* and *B* would contain the receptor.

Uemichi, working in Furuhata's laboratory in Japan, was unable to confirm Schiff's results, and contends that there is no group-specific antigen in group *O* cells. This fact is reconciled with the multiple allelomorph hypothesis of heredity by a somewhat similar hypothesis evolved by Furuhata, which will be discussed in chapter X.

Continuing Bernstein's hypothesis, from the side-chain theory it is postulated that in the tissue cells at the origin of the a-agglutinin, for example, so much of the A-agglutinogen will be supplied that an equilibrium process of reciprocal binding arises, which protects the red blood corpuscles. It is significant that at the first appearance of the agglutinins in the serum, which takes place later than the appearance of the agglutinogens, apparently spontaneous disarrangements of these internal regulatory processes may arise. Thus it has been pointed out that the time of convulsions in infancy coincides approximately with the time of the production of the agglutinins. In a study of this phase of the problem, however, I was unable to find convulsions occurring more often in group *AB*, or less often in group *O*, than in any other group.

The validity of these assumptions depending on the correctness of the multiple allelomorph hypothesis of heredity as applied to the blood groups, a fact not yet proven beyond all doubt, the final judgment in this matter must await further experimentation. In the meantime, for all practical purposes, we may accept the fact that there may be evident in human serums two specific isohemagglutinins, and in erythrocytes two corresponding and equally specific isohemagglutinogens. In addition, it may be accepted that such an agglutinin is not evident in a blood which contains the corresponding agglutinogen, and vice versa. On this basis every person (with certain exceptions which will be discussed in the next chapter) may be placed in one of the four blood groups by the proper technic.

There is little demonstrable difference between agglutinogens A and B except their relative frequency. Agglutinogen A, for instance, is found among European peoples several times as frequently as aglutinogen B, while agglutinogen B occurs more frequently than A among many peoples of the Orient (see chapter XIV). An additional demonstrable difference found by Schiff and Adelsberger (1924) is that red cells containing agglutinogen A are intensely agglutinated by some serums immunized against the "heterogenetic antigen" of Forssman. As for the agglutinins, like other antibodies they are known by their effects rather than by what they are.

CHAPTER III

The Serologic Basis for the Groups

"The methods generally employed for blood grouping are based upon the assumption that there are only four iso-agglutinin groups. . . . . The tests based on this assumption are so devised as to cause each blood tested to fall into one or the other of these groups, thus serving to perpetuate a belief which no one has seriously questioned."—Guthrie and Huck, *On the Existence of More than Four Iso-agglutinin Groups in Human Blood*, 1923.

INTRODUCTION

The division of human blood into four groups is not so simple as was once thought. There have been presented and elaborated exceptions of two sorts: first, a series of phenomena involving atypical reactions such as pseudo-agglutination, auto-agglutination, and "cold"-agglutination; and second, additions to the four original groups, in the form of sub-groups, due to the alleged presence in certain bloods of additional iso-agglutinating elements.

PSEUDO-AGGLUTINATION

The first class of exceptions (pseudo-agglutination and similar phenomena) has in addition to its scientific interest an added importance in that these conditions may, unless especial care is taken, cause mistakes in the typing of blood. Rouleaux formation, or the tendency of the red cells to adhere together in columns, like piles of coins, is one of the commonest of pseudo-agglutination phenomena. It was recorded as early as 1853, and has been a center of discussion ever since. Marked rouleaux formation might be mistaken for agglutination by the untrained worker, and in certain pathological disturbances where rouleaux formation is unusually heavy it is well to take precautions against errors in reading. In various diseases, such as rheumatic fever, tuberculosis, pneumonia, and cardiac diseases, and in certain physiologic conditions such as menstruation and pregnancy, the blood shows a high sedimentation rate, and a consequent tendency to pseudo-agglutination. The serum plays the important part here, having apparently an increased capacity to agglomerate the red cells of its own or other bloods. It was this tendency that Shattock saw in his oft-quoted work on the buffy coat in shed blood.

The increasing use of the sedimentation test in diagnosis in pregnancy and certain pathologic disturbances should make the characteristics of this phenomenon familiar enough to obviate any mistakes in blood grouping due to pseudo-agglutination. Definite precautions may be taken if necessary, however, by inhibiting the tendency to pseudo-agglutination or by destroying the agglomeration. It has been shown by Lattes (1924), Falgairolle (1926), and others that certain substances may be added to serum, depriving it of its rouleaux-forming properties, without affecting the iso-agglutinins. Lecithin is one of these substances. One part of kaolin suspension to three parts of serum will also prevent pseudo-agglutination. Formalin and hypotonic salt solution have likewise been suggested. Shattock found that pseudo-agglutination was stopped by dilution of the serum with plain salt solution. Mechanical agitation will break up the clumps, whereas this only causes stronger clumping in true iso-agglutination.

The intensity of the reaction in pseudo-agglutination is not less at 37°C. than at lower temperatures, and may be greater. The active substance in pseudo-agglutination can not be absorbed by treating with erythrocytes. In true isohemagglutination it can be so absorbed.

These facts make it important to take precautions against pseudo-agglutination in any case where its occurrence is suspected, as in the pathological and physiological conditions mentioned above. The use of lecithin or kaolin where any doubt occurs makes this possible.

"COLD"-AGGLUTINATION AND AUTO-AGGLUTINATION

Another phenomenon which may under certain conditions become a possible source of error in blood grouping is the occurrence of agglutination at low temperatures (mainly from 0 to 5°C.). Such agglutination usually diminishes as the temperature increases, and generally disappears at 37°C. When the individual's red cells are agglutinated under such conditions by his own serum, the reaction is spoken of as auto-agglutination. When the agglutination occurs due to serum of another individual, the reaction is known as "cold"-agglutination. These two reactions are certainly related and may be identical. A number of European workers consider this agglutination at low temperatures as a form of pseudo-agglutination resulting from quantitative variations in the properties of the serums or corpuscles. While it is true that certain apparent cases of auto-agglutination may be due simply to rapid sedimentation rate, there seems to be no doubt that "cold"-agglutination is generally due to definite agglutinins and agglutinogens acting only or mainly at low temperatures.

Landsteiner and his co-workers have shown that in auto-agglutination and other cold-agglutination, the active principle in the serum may be absorbed by the cells, thus showing it to be a true agglutinin. It is again set free by warming the cells. The agglutinin may thus be obtained in salt solution at room temperature, and its presence demonstrated by adding new cells to the fluid and lowering the temperature. Landsteiner goes so far as to suggest a specificity in the "cold" agglutinins corresponding to that of normal iso-agglutinins. Table 3 summarizes the distinguishing characteristics of the various types of agglutination considered in this chapter.

Auto-agglutination is greatly increased in certain pathologic disturbances. Among these have been listed hemolytic icterus, paroxysmal hemoglobinuria, trypanosomal and spirochetal infections, syphilitic or hypertrophic cirrhosis, Raynaud's disease, secondary and pernicious anemia, chronic leukemia, and pneumonia. Differential diagnostic significance has at times been attributed to the degree of auto-agglutination as found in these and other conditions (e.g., acquired hemolytic jaundice).

TABLE 3

DISTINGUISHING CHARACTERISTICS OF THE VARIOUS TYPES OF AGGLUTINATION

	EFFECT OF ERYTHROCYTES ON ACTIVE PRINCIPLE	INFLUENCE OF TEMPERATURE	EFFECT OF DILUTION OF SERUM
Pseudo-agglutination	Not absorbed	Somewhat more active at 37°C. than at lower temperatures	Reaction disappears
Auto-agglutination and other "cold"-agglutinations	Absorbed	Occurs mainly from 0° to 5°C.	Reaction remains
Iso-agglutination	Absorbed	Not affected by variations from 0° to 37°C.	Reaction remains

Under suitable conditions, such as cold working conditions, "cold"-agglutination might appear as a minor, atypical iso-agglutination reaction, confusing the typical grouping of bloods. This may account for some of the atypical reactions recorded by various workers. At ordinary "room temperature," however, such reactions will normally not be encountered, and need not interfere with the established four-group scheme.

SUB-GROUPS

The second class of exceptions to the four groups consists of those forming the so-called sub-groups. These exceptions are based on various anomalous reactions of certain serums and certain erythrocytes, and are held by their proponents to be due to true isohemagglutinins and agglutinogens acting in the same way and at the same temperatures as those resulting in the four established groups. Opponents of this view contend that there are but four groups and no more, referring the anomalous reactions to incomplete absorption or other errors in technic. There is a considerable body of evidence on each side of the question.

As early as the discovery of blood groups, exceptions to the general rule have been noted. Landsteiner himself called attention to some of these.

TABLE 4

RESULTS OF ABSORPTIONS OF GROUP *O* SERUM (AFTER GUTHRIE AND HUCK)

	GROUP *AB*	GROUP *B*	FIRST KIND OF GROUP *A*	SECOND KIND OF GROUP *A*
Group *O*	+	+	+	+
Group *O* after absorption with cells of group *AB*	0	0	0	+
Group *O* after absorption with cells of first kind of group *A*	+	+	0	+
Group *O* after absorption with cells of second kind of group *A*	+	+	0	0

Since then nearly all investigators have noted cases in which serums or cells did not behave as was to be expected, judging from their supposed group. By successive absorptions with cells of various individuals, all of the same group, certain serums have been shown to contain several agglutinins within one of the four traditional groups. Other serums have been noted which gave fewer reactions than were to be expected judging from their group based on the corpuscles. Correspondingly, erythrocytes have been found which contained agglutinogens not accounted for on the basis of the four main groups.

Guthrie and his co-workers were the first to study these exceptions in a systematic way, and to attempt to explain them. They called attention to the fact that the methods used for typing an unknown blood are based

upon the assumption that there are only four iso-agglutination groups and that the blood of every person belongs to one of the four. These methods of typing an unknown blood are so devised as to cause it to fall automatically and necessarily into one of the four traditional groups. Guthrie and Huck presented evidence to show that these methods were not adequate.

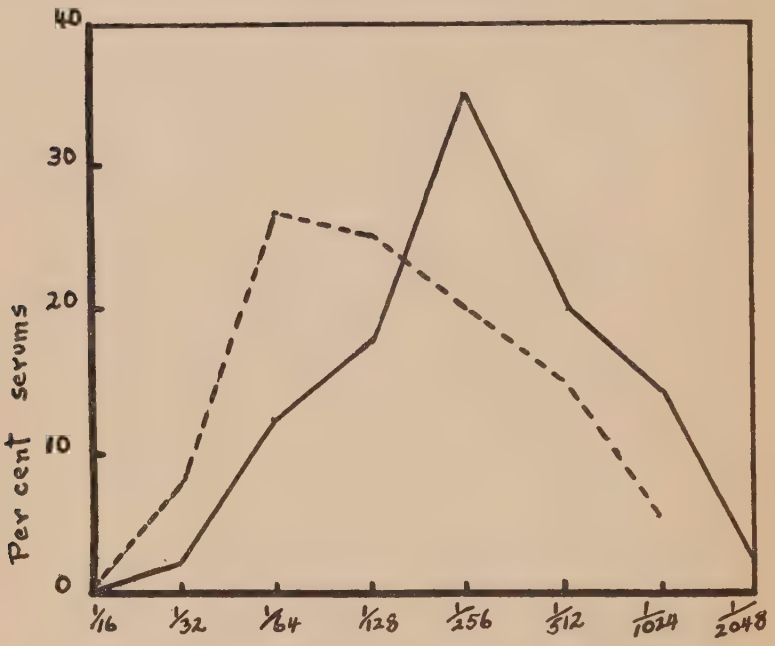


FIG. 1. VARIATION IN AGGLUTININ TITRE OF VARIOUS SERUMS

Agglutinin a represented by solid line, b by dotted line. Redrawn from Schiff and Mendlowicz 1926.

First of all it was found that the red cells of various persons of group *A* did not always act alike. For instance, group *O* serum was absorbed with erythrocytes of group *AB*. Theoretically, this should have removed all the iso-agglutinins from the group *O* serum, and it should no longer have been able to agglutinate the cells of any other group. As was expected, it was not capable of agglutinating erythrocytes of persons of groups *AB*, *B*, and of certain persons of group *A*. Contrary to expectation, however, it was still capable of agglutinating the red cells of other members of group *A*. Absorption of group *O* serum with erythrocytes of the first kind of group *A*

left the serum still capable of agglutinating cells of the second kind of group *A*. It appeared that in the second kind of group *A* bloods there was an additional pair of agglutination elements (see table 4). Guthrie and

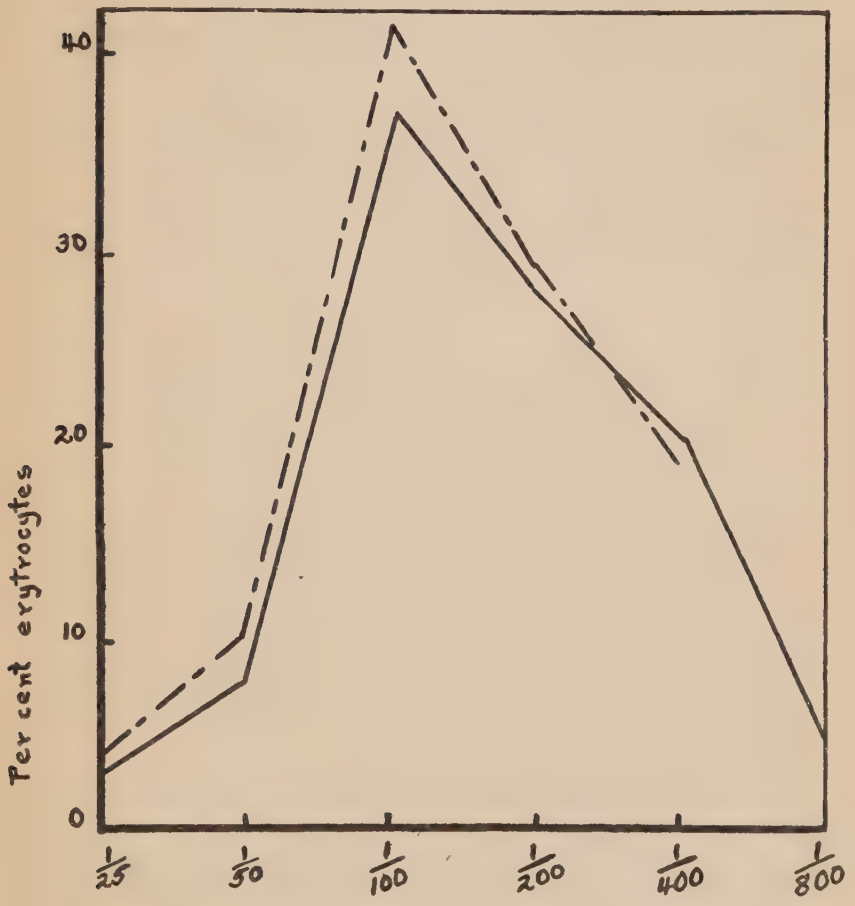


FIG. 2. VARIATION IN SENSITIVITY OF VARIOUS ERYTHROCYTES

Agglutinogen A represented by solid line, B by dotted line. Redrawn from Schiff and Hübener 1925.

Huck named these hypothetical new elements C and c, respectively, and tentatively estimated the incidence of the new type of group *A* erythrocytes at from 20 to 25 per cent. Later they were led to question this estimate, and the incidence was raised.

Within the last five years various workers have found additional agglutinating elements similar to, and probably identical with, those of Guthrie and Huck, and have reported the incidence of the new type of group *A* erythrocytes in varying but similar amounts (75 to 80 per cent). No careful work has been done on the inheritance of these hypothetical additional elements, but preliminary studies seem to indicate that they are not hereditary.

Further reports from Guthrie's laboratory have indicated the presence of still other agglutinins and agglutinogens. However, none of these has been studied as yet from all angles. Some investigation is indicated along the lines of differentiating the antigenic properties of the various anomalous types by means of animal immunization. The possibilities and limitations of this line of work have already been shown by Hooker and Anderson (1921) and others.

Later workers on the question of additional agglutinating elements have provided in their experiments quantitative controls for titers, which were lacking in the earlier work. The results of such work have provided one of the chief points in the objection to the postulating of new agglutinins and agglutinogens. It has been found by Mino (1924), Lattes (1924), and others, for example, that single absorptions are not always sufficient to exhaust a specific agglutinin or to satisfy an agglutinogen. Considerable differences in titer of different bloods are found, so that a single absorption leaves certain bloods unexhausted, giving differential responses which might be interpreted as indicating additional agglutinating elements, but which really only indicate incomplete absorption. This variation in agglutinating power of serums and agglutinophyllic capacity of cells has been established beyond question (see figs. 1 and 2). Whether or not it will explain the anomalous results is another matter.

A large number of workers assert that there are only four groups, and that all contradictory opinions are based on various sources of error, such as pseudo-agglutination, low titer, incomplete absorption, and other quantitative effects. Still other causes for the anomalous reactions have been suggested, such as the occasional atrophy of a physical quality of the serum, the hereditary partial injury of agglutinogen A, or a physiological condition paralleling a congenital deficiency of some anatomic structure.

Landsteiner and Witt (1926), however, were able to separate two qualitatively different fractions of agglutinins from serums *O* and *B*, the one acting on both types of group *A* cells, the other chiefly on one of them. This indicates the presence of true iso-agglutinins and of qualitative differences in the corpuscles. However, since there is as yet no indication that the new

type of group *A* cells occurs independently of the old type, it is not necessary to make any definite change in the standard scheme of grouping, but only to consider the possibility of sub-groups within certain of the four traditional groups.

One practical application of these studies stands clearly forth, however. Whatever the ultimate outcome of the question of additional groups, the present uncertain status makes it a practical necessity to perform direct matching tests immediately before transfusion in addition to determining the groups of donor and recipient.

CHAPTER IV

The Establishment and Subsequent Stability of the Blood Groups

> "Die spezifischen Differenzierungsprozesse im Serum, das Erscheinen normaler Antikörper ist ein Vorgang, der morphologischen Differenzierung des Organismus analog; wir können der Morphogenese die Serogenese gegenüberstellen. Nun ist der Vorgang der Serogenese bei der Geburt noch nicht abgeschlossen, und diejenigen Antikörper, die im erwachsenen Organismus nachweisbar sind, fehlen in der Regel den Neugeborenen."—Hirszfeld, *Ueber die Konstitutionsserologie im Zusammenhang mit der Blutgruppenforschung*, 1926.

ESTABLISHMENT OF THE GROUPS

It has already been stated that every person can be placed in one of the four major blood groups. The questions naturally arise how early in life the blood group can be determined, and whether once determined, there is any change throughout the life of the individual. In addition to their purely scientific interest, these questions have important bearings on practical problems, especially that of transfusion in children.

As early as 1900, that is, immediately after the discovery of the groups, the question of blood groups in infants was attacked, and it gave rise to a discussion which lasted for more than twenty years. Halban in 1900 studied the interagglutination of maternal and fetal blood, the fetal blood being from the umbilical cord at birth. He made the observation that maternal serums contained stronger and more frequent agglutinins than fetal serums, and that maternal erythrocytes were more readily agglutinated than were fetal erythrocytes. Halban also noted that there were often present in a mother's blood agglutinins which were not present in the infant's blood. This would seem to indicate that agglutinins did not pass through the placenta and thus become acquired by the fetus.

Various methods for the production of iso-agglutinins in human blood have been suggested, but none definitely proved. One suggestion is that just mentioned: placental passage. Other suggestions have been that they are absorbed by the infant through its mother's milk, where iso-agglutinins are known to occur; that they are the result of hereditary factors; that they are not the result of hereditary factors, but that all serums are alike; that they are formed by an immunity reaction similar to that producing bacterial agglutinins; and that they are formed by the disintegration of erythrocytes.

In regard to placental permeability, a great deal of work has been done. Transmission of antibodies of various kinds through the placenta has been affirmed by various workers (Schumacher 1901, Polano 1904, Bourquin 1921, Ruh and McClelland 1923, Kuttner and Rattner 1923, et al.), and denied by others Fellenberg and Döll 1913, Collon 1927, et al.). Hirszfeld and Zborowski (1925, 1926) have suggested a selective permeability of the human placenta for iso-agglutinins, varying in the four groups. They described a protective mechanism whereby when mother and infant were of different groups (heterospecifische Zwangerschaft) there was a tendency for the disappearance in the mother's blood of iso-agglutinins which might interact with receptors in the infant's cells. Rech and Wöhlisch (1926) and Collon (1927) disagree with such a hypothesis. Collon has demonstrated the non-disappearance in the mother of iso-agglutinins which could react with the corpuscles of the fetus. He believes that the fetus is capable of elaborating its own antibodies and that the iso-agglutinins of the mother are not influenced sensitively by heterospecific pregnancy.

The conflicting statements on placental permeability were explained by Kuttner and Rattner on the basis of variations in the structure of the placenta of the various mammalian groups used in the several experiments. The single layer of cells of the human or rodent placenta presents potentially different possibilities from the complex arrangement of the placenta of the ruminants.

The question of placental permeability will be discussed again in this chapter.

Subsequent workers following Halban found that iso-agglutinins were frequently absent from infants' bloods. Some investigators found no agglutination at all of a mother's corpuscles by her own infant's serum, and concluded that it was perfectly safe to use a mother's blood to transfuse into her own infant without any compatibility tests. Other workers, however, showed that iso-agglutinins, while usually absent at birth, are sometimes present in a greater or less concentration. Still more important is the observation that where iso-agglutinins are absent at birth they may become established at any moment during the first year or two of life. Jones was able to demonstrate agglutinins in a seven months fetus.

As to the receptors in the red cells, the agglutinogens, it is generally conceded that they are normally present at birth. Various investigators have demonstrated their presence in fetuses as early as four months. Thus infants may usually be assigned to a blood group by testing their red cells, although the serum might not show the complementary agglutinins. De Biasi (1923), although agreeing that infants' cells could be grouped by test

serums, found that in no case was there any agglutination between the blood of a mother and that of her new-born babe, regardless of the fact that they often belonged to incompatible groups. This agreed with some of the earlier work, making it seem unnecessary to use compatibility tests in transfusing blood into infants.

Happ (1920) in a very carefully worked out series of studies, found that the blood group in infants is rarely as yet established, but the percentage of infants in whom it is established increases with age, so that after one year the group is usually established in all children, and after two years is always present as in adults. He also confirmed the observation that the agglutinogens are established earlier than the agglutinins. These results are summarized in the appended table (table 5) from Happ.

TABLE 5

Relative Time of Establishment of Isohemagglutinins and Agglutinogens (from Happ)

AGE	NUMBER OF INDIVIDUALS	PER CENT SHOWING AGGLUTINOGENS	PER CENT SHOWING AGGLUTININS	PER CENT HAVING BLOOD GROUP FIXED
Birth to 1 month	49	65.3	14.2	16.3
1 to 3 months	18	55.5	33.3	22.2
3 to 6 months	22	45.4	36.3	31.8
6 to 12 months	23	65.2	65.2	69.5
1 to 10 years	32	56.2	87.5	96.8

Smith (1927), in a very careful study of iso-agglutinins in the newly born, observed that there were frequently present in the umbilical cord blood of an infant, iso-agglutinins which corresponded to the agglutinins in the mother's blood. Within ten days these agglutinins diminished in titer and disappeared. This is evidence that agglutinins are transmitted by way of the placenta, but that these are soon lost. This loss may be permanent. On the other hand, similar or additional iso-agglutinins may be subsequently acquired by the infant, and these later acquisitions determine its real blood group. Interesting examples of this phenomenon are summarized in table 6 compiled from Smith.

I have myself noted occasional cases where agglutinogens not present at birth were subsequently acquired, and of course a number of cases where agglutinins were acquired during the first few months of life.

It would seem, then, that the following summary is justifiable. Isohemagglutinogens are usually present in the erythrocytes at birth, so that

the blood may be grouped by means of test serums. Occasionally, however, subsequent acquisitions of receptors may change this tentative group. Permanent iso-agglutinins, on the other hand, are rarely present in the serum at birth, but may become established at any moment during the first year or two of life. It is possible in many cases, however, to demonstrate the presence in the serum from the umbilical cord, of iso-agglutinins corresponding to those in the mother's blood. These are antibodies derived directly from the mother through the placenta, and are soon lost (within a week or ten days). They have nothing to do with the true blood group of the infant. Later acquisitions of similar or new agglutinins will determine the final group. While agglutinins derived from the mother by way of the placenta may be lost during the first week of life, there is practically

TABLE 6

SELECTED CASES OF AGGLUTININS TRANSMITTED BY WAY OF THE PLACENTA AND SUBSEQUENTLY LOST (COMPILED FROM SMITH)

AGGLUTININS IN MOTHER'S BLOOD	AGGLUTININS IN INFANT'S BLOOD			
	Cord	9 to 14 days	Later during first year	Permanent
a	a	—	a, b	a, b
a, b	b	—	a, b	a, b
a, b	a	—	a	a
a, b	a, b	a, b	a	a, b
a, b	a, b	a	a, b	a, b
b	b	—	a, b	a, b
b	b	—	b	b
a, b	a, b	—	b	a, b

no evidence that later agglutinins, or agglutinogens, when once established, ever change. Such evidence as is found concerning change of groups will be discussed in the second part of this chapter.

There may be a selective permeability of the placenta in certain hereditary relationships of the blood groups of mother and fetus. The total absence of agglutinins in the serum of the newly born, however, can not be upheld. A baby's serum, therefore, may agglutinate the transfused cells of a donor of incompatible group, and a baby's erythrocytes may well be agglutinated by the serum of an incompatible donor of high titer. While in many cases no reaction would occur in the absence of compatibility tests, the fact remains that children may have the blood group definitely established. Thus the same careful procedure that is used in choosing donors for adults must be observed in preparing to transfuse infants.

STABILITY OF THE GROUPS

When once the blood group is established, does it ever change? The titer apparently rises during the first few years of life, remaining constant throughout the greater part of the individual's existence, but decreasing definitely after the age of fifty. The agglutinins and agglutinogens themselves, however, except for occasional cases of the exceptionally late establishment of group, do not seem to undergo any permanent change after infancy. A number of investigators about the year 1922 presented evidence that temporary changes in blood group might be brought about by etherization, Roentgen irradiation, or the administration of various drugs (quinine, calcium lactate, antipyrin, arsenic, ether, chloroform, and others). These reports have been carefully investigated since then, and no corroboration has been found. In a careful study, Mino (1923) states that from experiments with numerous patients and various drugs he finds it impossible to modify the reactions and change the group. Changes of agglomeration have been suggested as the cause of the alleged group changes.

Harper and Byron (1922) have reported a change in the B-agglutinogen due to dietary factors. This work has never been confirmed. Denissenko and Scheinermann (1927), by titration of test serums before and after exertion, found that the agglutinophyllic capacity of the erythrocytes is increased from 50 to 150 per cent by physical fatigue.

Thomsen (1926) reported the accidental discovery of a transmissible agent whose nature was unknown, which caused erythrocytes of any group to be agglutinated, that is, to react as group *AB*. It was later found by one of Thomsen's students that the reaction was due to a bacillus which sensitized the blood cells to an agglutinating agent in the serum. On this basis especial care must be taken when the red cell suspension to be tested is not perfectly fresh. If it appears that a higher per cent of *AB* bloods are being found than would normally be expected, control may be made with group *AB* serum, which contains no isohemagglutinins, and should of course not agglutinate the bloods. If they are agglutinated by group *AB* serum, it is evidence that some other agglutinating agent is at work.

Thomsen also found the percentages of the four groups in old people (65 to 102 years) to be similar to those in younger persons, indicating no change during lifetime.

Several investigators working with the malaria treatment of paresis have reported changes in the blood groups of patients after treatment. No changes could be observed in untreated paretics. Such changes are reported as constant. Table 7 shows the changes recorded by Feldmann and

Elmanowitsch (1925). No confirmation of these results has since been made.

In investigating the alleged changes in blood groups following various procedures, Shumacher and Atzerodt (1926) could confirm only the pseudo-agglutination which occurs after diathermy of the abdomen and after passage of a galvanic current through the body.

TABLE 7

CHANGES IN BLOOD GROUP AFTER TREATMENT WITH MALARIA (FROM FELDMANN AND ELMANOWITSCH)

NUMBER OF CASES	BLOOD GROUP BEFORE TREAMENT	BLOOD GROUP AFTER TREATMENT
4	*AB*	*B*
1	*A*	*AB*
1	*A*	*O*
2	*A*	Serum *O*, cells *AB*. Nevertheless, no auto-agglutination was observed
2	*B*	
5	*O*	

Bahl (1929) has recently reported the changing of blood groups in children following transfusion. In one case, the patient and donor were both group *O*, in another, both were group *B*. In both cases the recipient showed a change to group *B* following the transfusion. These results have been questioned by Raestrup (1929) and Poehlmann (1929) on the grounds of faulty technique.

Within the past few years there have appeared a number of papers all affirming the fixity of the groups. It would seem that the blood groups are remarkably stable under rather extreme environmental conditions.

CHAPTER V

The History of Transfusion

"Adsit juvenis robustus, sanus, sanguine spitituoso plenus: adstet exhaustus viribus, tenuis, macilentus, vix animam trahens. Magister artis habeat tubulos argenteos inter se congruentes, aperiat arteriam robusti, et tubulum inserat mumatque: mox et aegroti arteriam findat, et tubulum foemineum infigat. Jam duos tubulos sibi mutuo applicet, et ex sano sanguis arterialis, calens et spirituosus saliet in aegrotum, unaque vitae fontem afferet, omnemque languorem pellet."—Libavius, *Defensio Syntagmatis Arcanorum Chymicorum*, 1615.

From the present high pinnacle upon which blood transfusion stands safely ensconced as a therapeutic measure, we may cast our eyes in two directions. Behind us lies the historical background of transfusion, extending through recent facts and discoveries to older legends and disappearing in age-old speculation. Before us lies the future, problematical, but most interesting. Without a knowledge of the past, we can not adequately conceive the future, or even completely understand the present, so that we may profitably review the history of transfusion as a guide to further progress.

Although we can place very definitely the date of the earliest transfusion in man, speculation on the subject extends as far back as we care to go. References in the writings of the ancient Egyptians, the Romans, and others, indicate that the operation was at least conceived of, and enthusiastically recommended, long before it was actually demonstrated. The well-known transfer of blood from three youths to Pope Innocent VIII in 1492 is now generally believed to have been made by mouth, in the form of a draught prepared by a Jewish physician. The drinking of blood being an old idea, this instance can not be considered as a landmark in the history of transfusion. Very probably other instances of the confusion of transfusion and the drinking of blood occur in the earlier records. Even as late as 1854, Rimund recommended the drinking of fresh calves' blood, which he said operated by a sort of transfusion.

As early as 1615, however, a definite description of a technic for transfusion was made by Libavius of Halle. The original passage, which appears at the head of this chapter, has been translated as follows:

"Let there be present a robust healthy youth full of lively blood. Let there come one exhausted in strength, weak, enervated, scarcely breathing. Let the master of the

art have little tubes that can be adapted one to the other; then let him open an artery of the healthy one, insert the tube and secure it. Next let him incise the artery of the patient and put into it the feminine tube. Now let him adapt the two tubes to each other and the arterial blood of the healthy one, warm and full of spirit, will leap into the sick one, and immediately will bring him to the fountain of life, and will drive away all languor."

There is some doubt as to whether this experiment was ever actually carried out or was simply an idea. Since the conception of the circulation of the blood had not yet been set forth by Harvey, it is doubtful if it was actually carried out. Harvey's conception, formulated in 1616 but not actually published until 1628, made the first tangible contribution to the history of transfusion.

Sir Christopher Wren, Savillian professor in the University of Oxford, took advantage of the knowledge of the circulation by performing experiments on the injecting of various medicated infusions into the veins of animals. He used a slender quill fastened to a bladder containing the matter to be injected. A logical outcome of these experiments was the attempt to inject blood from one animal to another. Dr. Richard Lower tried such experiments on dogs, with great success, the chief drawback being that in order to transfuse one animal another was sacrificed, the donor being bled to death. In these experiments the carotid artery and jugular vein were used. Thus was the first successful blood transfusion in animals carried out at Oxford in 1666. Other experiments on animals were tried in various places (Daniel of Germany, King and Coxe of England, et al.).

Dr. J. D. Major, professor of medicine at Kiel, published in 1667 accounts of transfusion in man, and may have been the first to successfully perform the experiment. The credit is generally given, however, to Jean Baptiste Denys, of Montpellier, physician to Louis XIV, who on June 15, 1667, successfully transfused nine ounces of the arterial blood of a sheep to a young man suffering from repeated bleedings. Denys and Emmerez were able for the first time to conserve the donor as well as the recipient. They used at first calves, dogs, sheep, and horses, finally turning their attention to man.

Lower and King in November 1667 made the first successful transfusion in man in England, using, as had Denys, 9 ounces of the arterial blood of a sheep.

Denys continued his transfusions in man, sometimes as a curative agency, sometimes with the aid of volunteers purely as an experiment, with varied success. Meanwhile "one of the most celebrated controversies which has

ever agitated the professors of medicine" arose around the operation. The proponents of transfusion claimed a universal remedy, which would restore health, youth, and vigor, cure mental diseases, calm violent dispositions, and prolong life. The opponents denied this and claimed it dangerous and

FIG. 3. BLUNDELL'S TRANSFUSION APPARATUS

From Keynes, after Blundell, by permission of the Oxford University Press

often fatal. Fantastic predictions such as the growth of horns on a man after transfusion from a sheep, were made.

The whole thing was terminated by an experiment by Denys on an insane man in 1668, when, after a third transfusion, the man died. The result was the sentence of the court of the Chastelet, which provided that "no

transfusion should be made upon any human body, but by the approbation of the physicians of the Parisian faculty." Banned in France by this decree, in Rome by a similar decree, and elsewhere by public opinion, transfusion suffered a decline, being only sporadically referred to in the literature for the next hundred and fifty years. In 1667 Sprat, in his *History of the Royal Society*, said, in speaking of Sir Christopher Wren's work: "Hence arose many new Experiments, and chiefly that of Transfusing Blood, ... that will probably end in extraordinary Success." In 1812, Thompson, in a similar "History of the Royal Society" said, after giving a short history of transfusion: "The expected advantages resulting from this practise have long been known to be visionary." Thus has the transfusion idea been alternately approved and condemned to this day, as the proponents or opponents have gained the majority.

In 1818 a sudden revival of the waning interest in the subject was manifested, due to the publication of the researches of James Blundell, lecturer on physiology and midwifery at St. Thomas's and Guy's Hospitals. His first work was with dogs, and was undertaken because of the many distressing human deaths he had witnessed after hemorrhage when the flow of blood itself had been arrested. He conceived the plan of regaining strength for the patient by transfusion. In 1824 the results of a long series of researches on transfusion, the properties of blood, and the effects of its withdrawal, were published. Blundell described his rather crude apparatus in 1818, and further modified and improved it in 1824. It was the first technic to use the syringe principle for transfusion in man. The apparatus is illustrated in figure 3.

Blundell's work demonstrated that blood might be received in a vessel and passed through a syringe without rendering it unfit for purposes of life; it demonstrated that there was at least one practical application of transfusion, that of replenishing blood after hemorrhage; and it further demonstrated that less transfused blood was necessary to save life than has been lost through hemorrhage. Blundell recognized that the operation was not yet firmly established as a surgical procedure, but he had confidence in its ultimate recognition, as evidenced by his belief that ". . . . after undergoing the usual ordeal of neglect, opposition, and ridicule, it will, hereafter, be admitted into general practise."

Blundell's work did indeed renew interest in the operation, and before 1900 many monographs and collections of cases appeared. The cases ran into the hundreds, with succesful results in about half of them. Fatalities often occurring (usually blamed on the introduction of air bubbles), the medical profession remained divided in opinion. However, new methods and technics were being devised. Blundell's apparatus was improved along

two general lines. It was recognized that coagulation of the blood had always been one of the chief obstacles to successful transfusions. Bischoff, in 1835, took a great forward step by using defibrinated blood. Many technics were built upon this principle and many were the bizarre instruments used to check coagulation, among them egg beaters, hair seives, and wire whips. As late as 1914 Moss described a method of using defibrinated

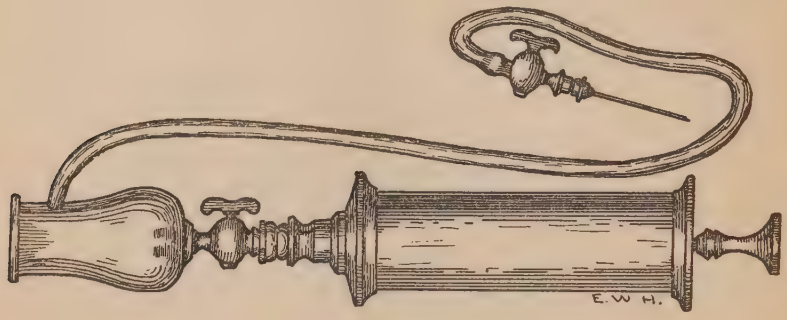


FIG. 4. GROSS'S TRANSFUSION APPARATUS

Redrawn from Gross 1866

FIG. 5. AVELING'S METHOD OF TRANSFUSION

Redrawn from Erichsen 1877

blood, and some use is made of these methods to the present day (see chapter VIII).

As a second method of overcoming coagulation, and the introduction of air bubbles, not involving defibrination, improvements were made in apparatus by various workers. Typical are Gross's instrument, in 1866 (fig. 4), and Aveling's method (fig. 5). Aveling's apparatus consisted of two

silver bevel-ended tubes, and an India rubber connecting tube with a bulb midway and a stopcock on each end. The bulb and tubes were first filled with warm water, then by alternately compressing one tube while the other is opened, and vica versa, the bulb being squeezed and filled meanwhile, blood is withdrawn from the recipient and injected into the donor.

Crile, in 1907, devised an effective method for direct transfusion. He made an anastomosis by drawing the recipient's vein through a small cannula and cuffing it back, then drawing the donor's artery over the vein and making it fast. This method is discussed in chapter VIII. It gave a wonderful impetus to the subject of transfusion, but was soon superceded by other methods due to its inherent difficulties, mainly the permanent scarring of the donor and the inability to measure the amount of blood given.

All these methods, including defibrination, were at best awkward procedures, and it was soon realized that indirect methods involving some way of preventing or delaying clotting, would be the only really feasible methods. The delaying of clotting was attempted by a variety of methods, such as paraffined tubes and syringes of various sorts, many of which are still in use. These methods will be described in chapter VIII.

The preventing of clotting offered another avenue of approach. Anticoagulants began to be suggested. Among those tried and abandoned were ammonia, ammonium sulfate, ammonium oxylate, sodium bicarbonate, sodium phosphate, sodium iodide, sodium sulphate, sulfarsenol, arsphenamine, and hirudin or leech extract. Salts of oxalic and citric acids were known to be anticoagulants, but were thought to be toxic. In 1914 it was shown by Hustin of Belgium that sodium citrate was an efficient anticoagulant and was not toxic in a dilution which was still effective. The first transfusion of citrated blood by Agote in Beunos Aires on November 14, 1914, marked the beginning of the present state of perfection of the operation. Weil (1915) and Lewisohn (1915) also applied the new method successfully.

In the meantime the discovery of the isohemagglutination reactions as described in chapter I had been made. Even with defibrination, and later with citrate anticoagulation, fatalities in transfusion cases had continued to occur. From the symptoms reported, we may be fairly certain that these were due to incompatibility of bloods. The discovery of the specific iso-agglutinins in the serum, and the corresponding iso-agglutinogens in the red cells, furnished the explanation of these fatalities. These discoveries, so all-important for the transfusion problem, lay practically unnoticed from their inception in 1900 until the exigencies of the World War brought the necessity for safe transfusions sharply to the fore. From then until the present day transfusion has been the chief practical application of blood grouping.

CHAPTER VI

Indications for Blood Transfusion

"The work of the recent past and the present on the physiology of the blood and connective tissues gives real promise for the future in the way of a control of the blood which shall mean eventually less and less dependence on transfusion as a palliative measure."—Doan, *The Transfusion Problem*, 1927.

HEMORRHAGE

The increased understanding of the blood groups and their application to transfusion, with the resulting advances in the safety of the operation, have brought about a rapid expansion of the clinical indications for transfusion. Most of these call for transfusion as a palliative measure. Only in one of the earliest of the indications, that of replacing blood suddenly lost from the circulation, can transfusion be considered as a curative measure. Even here the transfusion is only curative as far as the hemorrhage itself is concerned, and may not necessarily control the condition giving rise to the loss of blood.

Many types of acute hemorrhage have been successfully treated by blood transfusion. Chief among these are obstetrical emergencies. Numerous workers continue to report successful transfusions in cases of placenta previa, ruptured extra-uterine pregnancy, postpartum hemorrhage, and other sudden losses of blood in connection with child-birth.

In ruptured ectopic pregnancy, the blood in the peritoneal cavity, being usually sterile and with intact erythrocytes, (Thies 1914, Döderlein 1920), may if desired be used for the transfusion. It is collected into citrate solution and filtered through sterile gauze, then used immediately. An ovarian vein, if exposed, has been suggested for use; otherwise an elbow vein as usual. There are dangers connected with this method, however, which make it advisable to use a donor where possible.

In other obstetrical emergencies a donor must be used. Haselhorst (1928) has recently reiterated the great advantages of transfusion in obstetrical hemorrhages, urging promptness and sufficient amounts of blood (up to 1000 cc.) as the chief necessary factors.

The precaution of grouping the blood of all pregnant women well before the expected time of delivery, and having compatible donors available, as advocated by Titus (1920) and others, is a very wise one.

Hemorrhages other than obstetrical are also prime indications for transfusion. Loss of blood from severe injuries, post-operative hemorrhages, the rupture of large blood vessels causing the blood to flow into body cavities or to the outside, all result in conditions that are amazingly benefited by transfusion, which seems not only to replace the lost blood and provide functional oxygen-carriers, but actually to check the bleeding itself, by increasing the coagulability. The routine pre-operative determination of bleeding and clotting time may at times indicate transfusion as a preventive for post-operative hemorrhage.

Reports continue to be favorable for the treatment by transfusion of hemorrhages from gastric and duodenal ulcers. In the intervention of hemorrhages in typhoid fever, many successful transfusions testify to the importance of this measure. Pulmonary tuberculosis, dysentery, and inaccessible vascular erosions may under conditions of hemorrhage indicate transfusion.

HEMORRHAGIC DISEASES

Hemorrhages resulting from or associated with certain diseases ("hemorrhagic" diseases) are also indications for transfusion. In these cases the hemorrhage may be cured without the disease itself being controlled. In hemophilia, transfusion is a specific for the particular hemorrhage occurring at any one time (Unger 1919, et al.). Even a small amount of transfused blood will bring about hemostasis. The results, however, are not permanent, and a subsequent hemorrhage will have to be similarly treated. Thus it is to the advantage of a hemophiliac to know his blood group and have compatible donors available at all times. Ottenberg and Libmann, realizing that the tendency of hemophiliacs to bleed decreases with age, suggest that small quantities of blood be regularly injected into the veins of such individuals in order to bring them safely through the dangerous age.

Operations upon patients suffering from jaundice frequently result in hemorrhage, due to the lengthening of the coagulation time of the patient's blood in this disease. To prevent or control such hemorrhages, transfusion has proved beneficial. Again the transfusion is of value only for the particular hemorrhage, actual or potential, and its effect is only transitory.

Hemorrhages of the newly born are subject to life-saving treatment by transfusion. As early as 1908 Lambert reported the curing of a case of melena neonatorum by transfusion. Subsequent workers have testified to the value of this measure in the various hemorrhages of new-born infants. Such hemorrhages may be from the nose or mouth, from the umbilical cord, from injuries, as a result of circumcision, or from the gastro-intestinal

tract (morbus maculosis neonatorum). In all of these conditions, transfusion controls the hemorrhage. Intramuscular transfusion by way of the buttocks has been suggested in cases where the bleeding has not progressed far; 20 cc. of blood will be found sufficient. The other methods of transfusing infants, however (see chapter VIII), will probably be preferred by most operators, especially where the hemorrhage is becoming acute. In these cases larger amounts of blood will be necessary. Compatibility tests must be made before the operation, just as in the case of transfusion into adults, as has been pointed out in chapter IV.

Another hemorrhagic disease for which transfusion has been used is purpura hemorrhagica. In some cases beneficial results have been obtained, in others no benefit was evident. Bernheim, Ottenberg and Libmann, Unger, Peterson, Larrabee, Feinblatt, and others have reported cases, some of which were successful, others not. The number of platelets may be temporarily restored to normal by the transfusion, thus permitting normal hemostasis to occur. Although the bleeding time is greatly prolonged in thrombopenic purpura, the coagulation time is normal, so that transfusion has no opportunity to act in this case in its well-known rôle of increasing coagulability. Larrabee (1926) urges the superiority of whole blood to citrated blood in increasing the patient's platelet count. Krasso (1927) and Engel (1928) have recently reported successful treatment of thrombopenia by transfusion. Engel considers that the erythrocytes play the important part, tiding the patient over the period of danger until thrombopoiesis starts again.

SHOCK

Closely associated with hemorrhage is shock: surgical, traumatic, or toxic. Transfusion is beneficial in the treatment of shock because the fundamental indication is similar to that of hemorrhage: loss of blood from the active circulation. The dilation and increased permeability of the capillaries allow fluid to collect, and escape through the walls, and the resultant low blood pressure, lack of functional oxygen carriers, cardiac weakness, and decreased alkalinity of the blood cause the condition of shock. When given early, transfusion may be a life-saving measure. As in the case of postoperative hemorrhages, transfusion may be used either to prevent or to treat shock. It is most important to perform the transfusion within a few hours of the onset of shock, if shock has already occurred, as otherwise vital centers may be damaged.

A good deal of publicity has been given the use of gum acacia (6 per cent solution in 0.9 per cent sodium chloride) as a solution to be transfused into

the circulation. This was an emergency method devised by Bayliss and his co-workers during the World War, when time and blood were precious. It was determined at the same time by Keith (1919) that the diminution of blood volume in shock was comparable to that which attended severe hemorrhage. Thus a fluid to be injected into the circulation was essential. In the absence of sufficient available blood during war emergencies, normal saline solution was tried, but it was found that the loss through the abnormally permeable capillaries was so rapid as to make it of little value. The gum acacia solution was devised as an emergency substitute which would stay in the circulation, but all workers agree that whole blood is more valuable, bringing with it as it does functional oxygen carriers and normal alkaline buffers in addition to its bulk.

Robertson and Boyd (1923) recommend blood transfusion to combat toxic shock from severe superficial burns in children. Exsanguination transfusion has been successfully used by these workers. This method of transfusion will be discussed in chapter VIII. Riehl (1927) used transfusion successfully in the treatment of severe burns. Other workers have endorsed transfusion in such cases, while some (cf. Baldwin 1925) have warned of its limitations.

Sudden loss of blood from the circulation, then, from whatever cause, presents the ideal indication for blood transfusion, and is the only indication for which this therapeutic measure may be considered curative.

Aside from hemorrhages, indications for transfusion include primary blood diseases, secondary anemias, subacute systemic bacterial sepsis, acute sepsis, poisoning by carbon monoxide and similarly acting substances, general debility, and finally a number of miscellaneous conditions for which transfusion is a suggested but scarcely proven therapeutic measure.

PERNICIOUS ANEMIA

The induction of remissions in pernicious anemia has been one of the outstanding results of blood transfusion, but the use of transfusion in this condition has in the past year or two been relegated to a minor rôle. Following the work of Minot and Murphy (1926, 1927, 1928), liver feeding has become the chief therapeutic measure and transfusion is now indicated only as an emergency method when the patient is too weak to be put immediately on liver diet. The important part that transfusion has played in the past in the treatment of pernicious anemia deserves mention here.

This measure was, of course, only palliative, and in no sense was the disease cured, but the immediate results of transfusion were always strikingly beneficial, and in many cases repeated transfusions led to remissions.

In these cases the benefits of transfusion consisted of the increase of functional erythrocytes and consequently of hemoglobin, and the hematopoietic stimulus. Possibly there was an added hemotoxic effect. The red cell count following transfusion for anemia has been increased manyfold. Introduced erythrocytes may function for several months.

Transfusion, before the advent of liver feeding, was indicated in the early stages of the disease, as soon as the diagnosis was established. By giving repeated transfusions of relatively small amounts (under 500 cc.), with a short interval of from three to five days between transfusions, thus adding blood elements before there was a decided decrease, it was frequently possible to bring the erythrocytes and hemoglobin to a normal point. Yates and Thalhimer (1926) have recorded the case of a patient who received 113 transfusions in three years.

At present the status of transfusion in pernicious anemia is considerably modified. It is still the one procedure that can be successfully employed to quickly relieve the symptoms in these patients. It is still a valuable therapeutic agent in emergency cases, or to cause a constant temporary improvement. Some workers (cf. Emile-Weil 1928) are combining transfusions and liver feeding. Transfusion is yielding, however, to increasing knowledge, and it may be hoped that transfusion will be likewise gradually superceded as a palliative measure in other diseases.

ANEMIA OF PREGNANCY

Attention has recently been called to the hemolytic anemia of pregnancy as an indication for transfusion (Allan 1928, et al.). This acute condition responds promptly to transfusion after the uterus has been emptied.

LEUKEMIA

As a palliative measure in the leukemias, transfusion has proven less satisfactory than it did in pernicious anemia. Feinblatt (1927) reports two cases with no benefit in either. Because of a few successful cases the operation is considered by most writers as always being worthy of a trial. As a treatment for hemorrhages of the leukemias, transfusion is of course always indicated.

SECONDARY ANEMIAS

In the secondary anemias, transfusion is frequently employed as an aid to treatment. It is only an aid, however, and while it may serve in some cases to swing the balance, it is the search for and treatment of the funda-

mental causes underlying the trouble that play the important rôle. Harris (1928) warns against the unnecessary employment of transfusion in connection with surgical operations in secondary anemias. He states that transfusions are unnecessary in most of the severe cases, whether acute or chronic, to insure successful surgical operation and complete recovery of health.

Eisen (1927) states that some patients with cancer suffer more from the accompanying anemia than from any other symptom associated with malignant disease. In these cases transfusions have proven very beneficial, although great care must be taken to avoid hemolysis.

MALNUTRITION IN INFANTS

The work of Fischer, Kerley, Leebron, Hess, Marriott, and others in this country, followed by that of Opitz in Germany, has established transfusion as a routine measure in treating premature and malnourished infants. Bakwin, Astrowe, and Rivkin (1927) in an attempt to ascertain the mode of action of the transfused blood in the treatment of severe malnutrition in infants, found that the injected plasma left the circulation shortly after injection, taking with it the introduced plasma proteins. The injected erythrocytes remained for "a considerable length of time" in the circulation, causing a corresponding persistent increase in the hemoglobin content. Likewise a persistent increase in the iso-agglutinin titer of the serum of the recipient was noted.

SEPTIC CONDITIONS

Conditions of sepsis often present indications for transfusion. Chronic septic conditions respond to transfusion, if at all, because they are usually accompanied by a condition of anemia. Reinhold and Kramer (1927) report excellent results of transfusion in children with septic affections. Lillie (1928) recommends blood transfusion and the intravenous injection of dye germicides as a supportive therapeutic measure in conjunction with surgical intervention in cases of general sepsis secondary to suppurative disease of the temporal bone. Feinblatt records twenty-one cases of subacute bacterial endocarditis with positive blood culture for Streptococcus viridans, all of whom were given transfusions from non-immunized donors. The results were negative and the patients all died. Kurtz and White (1929) treated such a case with blood from a donor immunized with a killed culture obtained from the patient's blood. The result was likewise negative and this patient also died.

In acute sepsis, many writers are of the opinion that the transfusion of

plain normal blood is not only useless, but even harmful (cf. Doan 1927). Other workers find successful use for the measure. Feinblatt reports successful transfusion in sinus thrombosis complicating mastoiditis, in puerperal sepsis, and in perforated gangrenous appendix. The general dissatisfaction with the results of this treatment, however, has brought into prominence the use of immunized donors. In this method donors are used who have previously been vaccinated with the organism obtained from the patient. Fry (1920), Stetson (1924), Linser (1925), Howell, Portis and Beverly (1927), Manoussakis (1928), and others, report good results with the use of immunized donors in bacteremia. The method of immunizing donors is given in chapter VII.

Unfavorable results from immuno-transfusions are occasionally recorded (Waldman and Kahn 1926). Instead of vaccinated individuals, persons convalescing from the disease in question are sometimes used as donors. This method has been especially used in typhoid and measles (Ribadeau-Dumas and Bressand 1918, Zingher 1924, Macnamara 1926, et al.). Schottmüller (1926) recommends this method for various infectious diseases. Hänsch and Hartmann (1927), however, while recommending transfusion in the treatment of typhoid, found no apparent difference in the results from using blood of typhoid convalescents, persons immunized against typhoid, or of non-immunes. The subject is one of active experimentation at the present time, and opinion will doubtless be modified freely in the near future.

CARBON MONOXIDE POISONING

In cases of poisoning by substances having their primary effect upon erythrocytes, blood transfusion has proven of value. Chief among such substances is illuminating gas, in which the carbon monoxide forms with the hemoglobin a relatively stable compound, carboxyhemoglobin. The resultant paucity of functional oxygen carriers is an indication for transfusion. In these cases transfusion is usually combined with the withdrawal of blood, thus providing another important use for exsanguination transfusion. Poisoning by substances such as benzol and nitrobenzol, having an effect similar to that of carbon monoxide, may likewise indicate transfusion.

GENERAL DEBILITY AND MISCELLANEOUS INDICATIONS

General debility may indicate transfusion as a palliative or preoperative measure. In addition to the indications mentioned above, all of which have been tested to some extent although not all of them sufficiently, there are a number of miscellaneous indications for which a single case report or a few

scattered reports stand sponsor. Thus, Bell (1920) recommends transfusion in eclampsia. Kerley (1924) cites beneficial results in cases of epidemic encephalitis. Hollander (1927) successfully used transfusion in a case of pemphigus. Low (1928) recommends transfusion of whole blood during or immediately after an attack of blackwater fever, in combination with the usual methods of treatment. Cole (1911) reported the successful use of transfusion in advanced cases of pellagra.

Diabetic coma has been suggested as an indication for transfusion. In 1915 Carlson and Ginsberg performed transfusion experiments upon dogs from which the pancreas had been removed. The results indicated the beneficial effects of blood transfused from non-diabetic dogs. Experiments in cases of diabetes, especially diabetic coma, in human beings have not met with such success (Raulston and Woodyat 1914, Ottenberg and Libman 1915, Garbat 1919), although Feinblatt and Sherman (1925) reported a case in which striking improvement was obtained. In this case the transfusion was combined with large doses of insulin, and the patient, who was in deep coma, immediately came out of coma, improved, and left the hospital within a week.

Schaffer and Rothman (1927) suggest transfusion in the treatment of erysipelas. They report a series of cases in which the mortality in the treated group was distinctly lower than that in the control group. Bass (1925) finds that the occurrence of pneumonia in anemic children is a definite indication for transfusion, in spite of the fact that pneumonia under other conditions has been considered a contra-indication. Flandin and Tzank (1925), Feinblatt (1926), and others, report occasional favorable results in the treatment with transfusion of nephritis and its associated hemorrhagic tendencies.

Lindemann (1919) successfully used transfusion in the treatment of tropical sprue. Manson-Bahr (1929) reports brilliant results from transfusion in treating sprue anemia. Immediate and apparently permanent benefits result even in patients who appear to be moribund. He considers the hematopoietic stimulus as more important than the mechanical replacement of the destroyed erythrocytes. The full benefits of transfusion in such cases are only obtained in conjunction with strict dietetic measures, and treatment with solution of potassium arsenite.

McBroom (1927) found success in five cases of bronchial asthma treated with transfusion. Among other more usual indications, Sidbury (1917) lists influenza acidosis, with fourteen cures in fourteen cases.

CONTRA-INDICATIONS FOR TRANSFUSION

Reilly (1927) lists the following contra-indications for blood transfusion: Acute cardiac disease, arterial sclerosis, pneumonia, hypertension, and any condition having cyanosis as a manifestation.

In this connection it is well to mention that Bass (1925) has found that pneumonia in anemic children is not a contraindication, but a distinct indication. Flinn (1929) offers a case of pneumonia successfully treated with transfusion as evidence that this treatment is devoid of danger and is a valuable form of therapy.

Duke (1926) has stated that from his experience in over 700 transfusions the only contra-indications were brain hemorrhage and allergy.

Cattell (1928), summing up transfusion for the International Clinics, gives the following contra-indications for transfusions:

1. Cardiac decompensation.
2. Severe respiratory embarrassment not ascribable to blood loss.
3. Suspected thrombophlebitis.

CHAPTER VII

Choosing a Donor; the Technic of Blood Grouping

". . . . there was the glamor of novelty and the feeling of satisfaction following an act of conscious heroism,—for such the sacrifice of blood was held to be, the days having long been forgotten when as much blood was 'let' in the treatment of almost any ailment."—Keynes, *Blood Transfusion*, 1922.

INTRODUCTION

From the interaction of the groups as outlined in chapter I it can be seen that many of the earlier reactions and fatalities following blood transfusion were due to agglutination of erythrocytes within the blood vessels of the patient. Although an important result of blood transfusion is the restoration of the bulk of the circulation fluid, an equally important function is the providing of red cells for carrying oxygen. The plasma of the donor is so diluted that its agglutinating power for the recipient's cells is relatively unimportant in most cases. On this basis it is usually regarded as sufficient that the plasma of the recipient should not agglutinate the cells of the donor. Thus group *O* is known as the "universal donor," since its erythrocytes are not agglutinated by the serum of any group. However, as outlined in chapter III, group *O* individuals may occasionally occur whose serum is of especially high titer. Under such conditions the plasma of the donor might be of importance in subsequent reactions.

Wichels and Lampe (1928) consider that agglutination of the recipient's erythrocytes by the donor's serum may take place in high grade anemia due to the small number of erythrocytes in the circulation of the recipient.

CHOOSING A DONOR

From the foregoing discussion it may readily be seen that the choice of the proper donor is the most important preoperative safeguard of transfusion. This involves attention to the physical, physiological, pathological, and even psychological qualities of the various available donors, as well as the careful routine examination of their blood, including grouping and direct matching tests.

The general consensus of opinion is that men are to be preferred to women as donors. Women are more inclined to anemia, and the veins are apt to be small and buried in fat. It has been noted that women donors recover their

cell volume and hemoglobin more slowly than men. The availability of a large superficial vein such as is more often found in men makes the operation easier for both physican and donor.

The best age is roughly from twenty to thirty years, although this may be increased to forty-five years if necessary. In older men the danger of inadequate blood regeneration increases.

The health of the donor is of prime importance. Syphilis, malaria, measles, small-pox, bronchial asthma, and other diseases have been transmitted by transfused blood. This means a general health examination where time permits, and in any case a Wassermann test should be made. Too much precaution can not be taken in this connection.

The hematologic status of the donor is considered an important point, and a study of the quality of the blood of prospective donors is always recommended, necessitating a minimum of a red cell count and a hemoglobin reading. Feinblatt makes the point that some subjects who offer themselves as donors show on examination a more marked anemia than the patient to be transfused. There are cited in this connection three very anemic professional donors with hemoglobin readings of 30, 40, and 70 per cent, respectively, with retinal hemorrhages present in one case.

Other cases of professional donors with hemoglobin readings below 85 per cent and red cell counts as low as 2,000,000 are on record.

In general the repeated giving of blood by a donor does not lead to anemia. A healthy man can quickly make up large losses of fluid. The total blood averages about one-twentieth of the body weight. This means that the usual removal of 500 to 700 cc. would be only about one-eighth of the total blood of an average man. However, occasional secondary anemias do develop in donors, and must be watched for. It seems to be well established that an average man may safely donate 500 cc. of blood every four to six weeks. Some do much more than that with apparent impunity. As much as 1400 cc. has been taken at one time from a donor, but this is not a wise practice.

A feeling of faintness may be noticed by the donor due to the loss of blood, or he may even actually faint due to the sight of blood or the thought of the operation. It is wise to keep the donor on his back for a while after the operation. An hour, or even less, may be sufficient.

A reflux of blood from the recipient to the donor may cause an infection of the donor. For this reason apparatus should be chosen which is designed to prevent this (see chapter VIII). The withdrawal of too much blood from the donor may cause characteristic reactions, such as yawning, sighing, sweating, pallor, increased pulse and respiration rate, and even collapse. Recovery takes place rapidly if the transfusion is discontinued.

It would seem that in some conditions, particularly pernicious anemia, patients receive much more benefit from certain donors than from others. This has been noted for years. Recently Kilgore, in discussing a paper on anemia, reported a case of a physician suffering from pernicious anemia, who received only very transitory benefits from 55 transfusions until finally a donor was found whose blood was extremely beneficial, and resulted in complete relief. This type of benefit seems to be independent of hemolgobin content and other determinable factors.

In cases of transfusion for some specific infectious disease, it has been suggested that the use of a donor who is convalescing from the particular disease, or who has recovered from it, is beneficial. Scarlet fever, poliomyelitis, typhoid fever, and especially measles, have been treated in this way. In the case of measles, Zingher (1924) has shown that whole blood injected intra-muscularly will prevent or modify an attack after exposure. Such cases have led to the use of immunized donors. Some promising results have been achieved by whole blood transfusions from donors who have been given a preliminary vaccination with the organism isolated from the patient.

With regard to the practice employed, the procedure follows in general that used in ordinary vaccine therapy. An initial subcutaneous dose of about one hundred million killed organisms is given, followed by further doses, the increments and intervals of which must be gauged by clinical conditions, care being taken to avoid an injection during the negative phase (Feinblatt 1926). Intervals of from four to seven days have been reported as most satisfactory. Determination of the titer of the immune bodies in the donor's serum is important before using his blood for transfusion, and the transfusion should be given when the phagocytic index is high.

While some cases of this nature have apparently been worth while, other results by this method have been indifferent, and a great deal of experiment is necessary to establish the procedure. Similarly, the use of alkalinized donors has been suggested for blood transfusions in acidosis therapy.

The psychological effects of transfusion on the donor can not be overlooked. Donors offer themselves from various motives, ranging from the purely altruistic urge of a stranger in an emergency, through the fraternal feeling of a member of the family, to the purely mercenary motives of the professional donor. During the war the unofficial reward of a leave of absence was a powerful stimulus to the donation of blood. In private cases the only reward outside of actual money paid is the feeling of self-sacrifice or the taste of adventure. Too much of this feeling will not only influence the donor's behavior during the operation, causing trouble for the operator,

but may even affect his future state of mind. Nervousness, excitement, and fear on the part of the donor should be overcome before the operation, or such a donor rejected where possible.

From this standpoint professional donors make the best subjects. This class of donors, listed in large numbers with every hospital of any size in this country, and with various organizations (Red Cross, etc.) in other countries, is rapidly growing. The danger is that the available lists will grow too rapidly, bringing transfusion down below the careful surgical plane it should occupy.

Professional donors are of known group; they soon learn their part of the technique and do not cause the operator any trouble; and they are available on short notice. Although the blood group need only be taken once, it is important to make the routine examination and especially the Wassermann test each time.

Brem (1928) recommends that donors fast for a certain period of time (overnight or longer) before the operation, in order to prevent febrile reactions following transfusion. When repeated transfusions are to be made into the same recipient, as for example in pernicious anemia, it is preferable to use a different donor each time, to prevent anaphylactic reactions.

Last of all, but perhaps most important, is the blood group of the donor. The blood of the recipient, and of the prospective donors who have withstood the tests just outlined, should be carefully grouped according to the methods described below. A donor should be chosen from the same group as the recipient. If no donor of the same group is available, choose a "universal donor" (group *O*), whose corpuscles can not be agglutinated by the serum of the recipient in any case, and whose serum will be so diluted in the recipient's circulation as to minimize the chances of its agglutinating the recipient's erythrocytes. To avoid donors of anomalous reactions, direct compatibility tests with blood of donor and recipient must be made immediately preceding the operation. Direct matching alone, however, must never be depended upon if it can be avoided, as the results by themselves are not always sufficient guides to the fitness of the donor. The titer of the iso-agglutinins varies not only among different individuals but even from time to time in the same individual.

The use of a donor of an incompatible group will cause severe reactions in the recipient. Donors of an incompatible group are still occasionally used, due to carelessness, staleness of serums, mistakes in technic of grouping, or emergencies. Typical of the reactions are the following: falling pulse rate, loss of consciousness, hemoglobinuria, pains all over the body, especially in the lumbar region, and heavy and labored breathing. Fullness in the head

is often complained of, followed by pains becoming localized in the lumbar region. Suffusion of the face and the sudden drop of the pulse rate are usual, and a severe chill, followed by high fever with temperatures of 103° to 105°F., is characteristic. Hemolysis following the agglutination reaction in the recipient's circulation results always in hemoglobinuria. Other symptoms may be urticarial rash, jaundice, and even delirium.

Death usually follows if more than 100 cc. of incompatible blood has been introduced. Since the reactions begin to appear early in the transfusion, the operation can usually be stopped before it is too late. If less than 100 cc. of incompatible blood has been introduced, recovery may occur. The subcutaneous administration of epinephrine (1 cc. of 1:1000) and atrophine sulphate (1/100 grain, or 0.000648 grams) will be found of value in this connection.

TECHNIC OF GROUPING

We assume that the agglutinating reaction is as accurate and specific *in vitro* as *in vivo*. Tests to determine the group of an unknown blood are thus built on the criteria of speed, convenience, and lack of potential sources of error. Early technics included cross-agglutinations of the serums and cells of donors and recipients, using glass plates and capillary pipettes. Since 1911, when Moss pointed out that only the cells or serums of known groups *A* and *B* are necessary to determine the blood group, the reactions looked for have been limited to two for each unknown blood.

The usual method is to use two test serums, of known groups *A* and *B*. Various methods of mixing serum and corpuscles have been worked out. Both macroscopic and microscopic readings have been used. The proponents of the macroscopic reading urge that with this method there is no danger of mistaking pseudo-agglutination for true agglutination. However, from the standpoint of speed and convenience the microscopic methods are to be preferred, and my own experience as well as that of others indicates that the results, when carefully arrived at, are perfectly dependable.

The method recommended by the medical section of the National Research Council provides a rapid and accurate technic. For this method test serums of known groups *A* and *B* are used. Such serums may be purchased from the various laboratories and supply houses, or they may be prepared from persons of known group and known titer. To prepare test serums, of which only small lots are necessary due to the dangers of using old serums, draw 10 to 25 cc. of blood from an elbow vein of a person of known group *A*, for example, with a syringe, and place it in a centrifuge tube to clot. Before drawing the blood the usual aseptic precautions of cleansing the skin must

be observed. After a few hours the clot in the centrifuge tube may be gently loosened, and the clear serum obtained by centrifuging. After pipetting off the supernatant serum, it may be kept by placing it in small glass capsules and sealing the ends in flame, or by other aseptic methods.

Test serum will retain its agglutinating qualities for months, if kept sterile. Vincent (1918) found no apparent diminution in the activity of serum one year old which showed a moderate degree of contamination. However, unfortunate experiences with old serums have led many investigators to urge the use of fresh preparations. Such an experience was that of Schumacher and Atzerodt (1926), who made a test infusion of 5 cc. of blood into a woman of group *O* from her son, whose group was also determined to be *O*. The result was an almost fatal collapse. By using fresh serums, however, the son was found to belong to group *A*. These investigators urge the necessity of checking test serums every two weeks.

Dried serum was once recommended, but it is not considered serviceable. Various methods of desiccation were tried, including drying on cover glasses and on filter paper. Karsner and Koekert (1919) found, however, that human iso-agglutinins showed deterioration in from one to three weeks after drying, regardless of the method of desiccation employed, or the previous addition of a preservative, or the elimination of salts by dialysis. These workers also found that such serums not only completely lost their group specificity, but acquired a non-specific agglutinating power for the corpuscles of all groups in from seven to ten weeks. This fact was taken by the writers to indicate the incorrectness of the assumption of specific agglutinogens in the red cells, a conclusion not borne out by subsequent experiments on animal immunization.

Various preservatives for test serums have been suggested, such as tricresol, phenol, quinosol, formalin, and others, but their use has been shown by Breitner to influence titer: an important point in view of the dispute over accessory agglutinins. Bacterial contaminations and filtration may also affect titer. Storage in sterile glass capsules, ampoules, or vials, and frequent checking against cells of known group are the necessary precautions to be taken with test serums.

In addition to the prepared serums, fresh cell emulsions from each person to be tested are necessary. The cell emulsion is made by allowing a drop of blood from the finger or ear to fall into a tube containing 3 cc. of isotonic saline solution. This makes an emulsion of suitable strength. If the cells are to be kept more than a few hours before grouping, it is best to add 1 per cent sodium citrate to the saline solution before making the emulsion. When the test is to be made, the cells are centrifugalized, the supernatant fluid pipetted off, and the fluid replaced with isotonic saline solution.

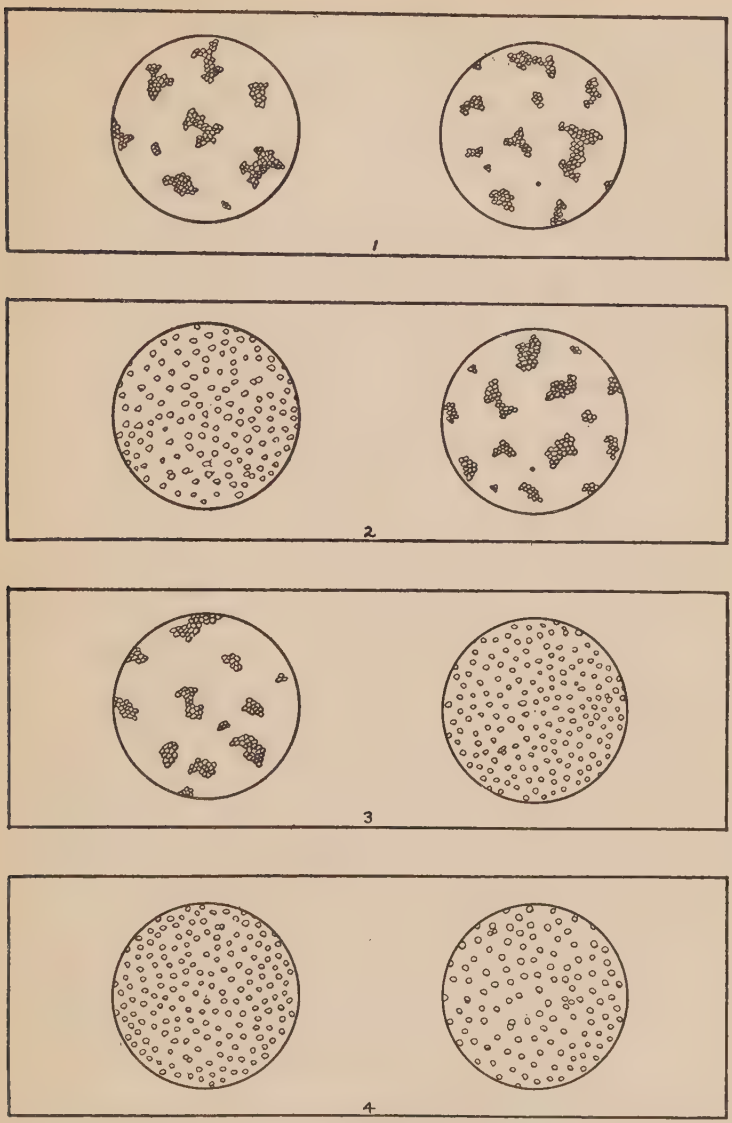


FIG. 6. DIAGRAMMATIC APPEARANCE OF THE RED CELLS UNDER THE MICROSCOPE IN EACH OF THE FOUR BLOOD GROUPS

Serum of group *A* on the left, serum of group *B* on the right. Slide 1, group *AB;* slide 2, group *A;* slide 3, group *B;* slide 4, group *O.*

Having at hand serums of groups *A* and *B*, and cell emulsions from the various persons to be grouped, the test is made as follows. A drop of group *A* serum is placed on the left hand end of a slide, and a drop of group *B* serum on the right hand end. Into each serum a drop of cell emulsion from the person to be tested is allowed to fall. The slide is tilted and rotated gently so that the cells are uniformly distributed; this is repeated every two minutes. A cover slip is placed on the mixture after two or three minutes of thorough mixing. The slides are examined under the microscope. At room temperature agglutination shows up plainly in from one to ten or fifteen minutes. Irregular clumps of cells, like brick dust, often visible to the naked eye, are the evidences of agglutination.

Room temperature (about 20°C.) is undoubtedly the most advisable temperature at which to perform grouping tests. As the temperature decreases, the possibility of observing "cold"-agglutination increases. After the reading has been made at room temperature at the end of fifteen minutes, it may be advisable to place the slides in the incubator at 37°C. for an additional half an hour to an hour, to obviate any chance of missing a weak agglutination due to low titer. This precaution is seldom used in the practical routine typing of bloods, however.

In reading the tests, the following rules will determine the group. If neither test serum agglutinates the cells, the blood belongs to group *O* (Jansky group I). If only the serum of group *B* agglutinates the cells, the blood belongs to group *A*. If the cells are agglutinated only by the group *A* serum, the blood belongs to group *B*. If both serums agglutinate the cells, the blood belongs to group *AB*. The macroscopic appearance of the cells in each of the four groups is shown in colors in the frontispiece, and the appearance under the microscope is shown diagrammatically in figure 6.

The above technic will be found very satisfactory, although some workers (Wolff 1924, Gram and Thomasen 1926, et al.) recommend a higher ratio of serum to cells. I have found in my own experience that the use of a large platinum loop is convenient instead of the use of droppers. In grouping large numbers of bloods at one time, I sometimes used a loop for the cells, flaming it each time, and individual capillary droppers for the test serums. The important point to be remembered in grouping bloods is the use of fresh sterile serums of known high titer.

White cell compatibility may in the future have to be taken into consideration, as Doan (1926) found that the serums of certain persons exerted a marked toxic effect on the white blood cells of certain others. Platelet studies from the standpont of group agglutination have thus far given negative results (Toda 1923), but these results need amplification.

CHAPTER VIII

The Technic of Transfusion

"Zuerst werden eine ganze Menge Methoden und Instrumente erfunden, auf welche die Erfinder nicht wenig stolz sind; dann gelangt man allmälig zu den richtigen Indicationen für die Operation, erkennt nun viel besser, was eigentlich geschehen muss, wirft das ganze Instrumentarium über Bord und behält aus demselben nur das Einfachste und deshalb Brauchbarste für die alltägliche Anwendung. Da ich annehme, dass die Transfusion aus ihren Kinderschuen herausgetreten ist, oder recht bald heraustreten wird, so schwärme ich keineswegs für neue Erfindungen von Spritzen, Canülen, Troicarts, Schröpfköpfen u.s.w."—Hueter, *Die arterielle Transfusion*, 1870.

Sifting through the methods for blood transfusion, which, with their numerous modifications and varying forms of apparatus, are legion, we find that there have been suggested and used four main types of procedure. The first is direct transfusion, by anastomosis of an artery or vein of the donor with a vein of the recipient. The three remaining methods involve indirect transfusion, in which blood is drawn from the donor into a vessel or syringe in which clotting is prevented or delayed, and then injected into the recipient. Three ways of preventing clotting have been used: defibrination, speed or smoothness of operation, and the addition of anticoagulants. Methods involving anticoagulants have been practically reduced to the various modifications of the citrate method. The four main methods of transfusion are thus as follows: (1) direct transfusion, (2) indirect transfusion with defibrinated blood, (3) indirect transfusion with whole unmodified blood, and (4) indirect transfusion with citrated blood. At the present time blood is always obtained from a vein of the donor. The method of injection into the recipient varies. The usual method is to inject by the intravenous route, but many other routes are used in special cases. The various methods and routes will be discussed separately and in some detail.

DIRECT TRANSFUSION

The anastomosis of blood vessels, a direct descendant of the original "animal to man" transfusions, was an exceedingly difficult operation, resulting in the infrequent use of transfusion until Crile (1906, 1907) found an effective method. This, the first practical procedure for the performing of transfusion from man to man, gave the operation a surgical standing and a

powerful impetus which resulted in the rapid modification of technic leading to the present efficient methods. Crile made the anastomosis by drawing the recipient's vein through a small cannula and cuffing it back. The donor's artery was then drawn over the vein and made fast, thus insuring a continuous unbroken passage for the blood. The radial artery at the wrist, and the median basilic or median cephalic vein at the elbow, are the ones used in this method.

Various modifications of this technic have been proposed (Elsberg 1909, Berheim 1912, Pope 1913, et al.). Elsberg's cannula is shown in figure 7. Direct transfusion has not proven satisfactory, however, and has been practically entirely discarded. The only advantage that can be cited for this method is that it delivers blood to the recipient practically unchanged from its condition in the donor. In the light of recent research on the effects

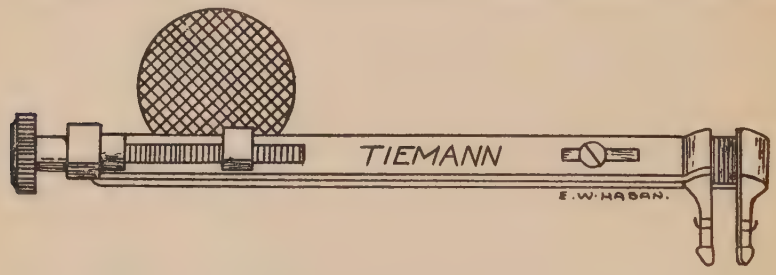


FIG. 7. ELSBERG'S CANNULA

Redrawn from Elsberg 1907, by permission of the *Journal of the American Medical Association.*

of indirect transfusion of whole unmodified blood and citrated blood, this advantage no longer outweighs the disadvantages of the method. The disvantages are as follows: it is not possible to measure accurately the amount of blood given; it means that the donor will be permanently scarred and that the artery must be ligatured at the close of the transfusion; it can not well be done with due regard to the condition of the patient; and finally, it is an operation involving extremely difficult technic.

INDIRECT TRANSFUSION INVOLVING DEFIBRINATED BLOOD

This technic (Bischoff 1835, Esmarch 1877, et al.) was the first suggested to overcome the dangers of coagulation. Before the advent of anticoagulants it was a relatively satisfactory technic. At the present time it is almost entirely superseded by other methods, although here and there it is still used with apparently satisfactory results (Opitz 1924, Platt 1924, Denecke 1926, Plehn 1926).

INDIRECT TRANSFUSION INVOLVING WHOLE UNMODIFIED BLOOD

Two sets of methods have been suggested and elaborated for the injection of whole unmodified blood. One set involves the use of paraffined tubes

FIG. 8. THE KIMPTON-BROWN TUBE FOR TRANSFUSION

Redrawn from Kimpton and Brown 1913, by permission of the *Journal of the American Medical Association.*

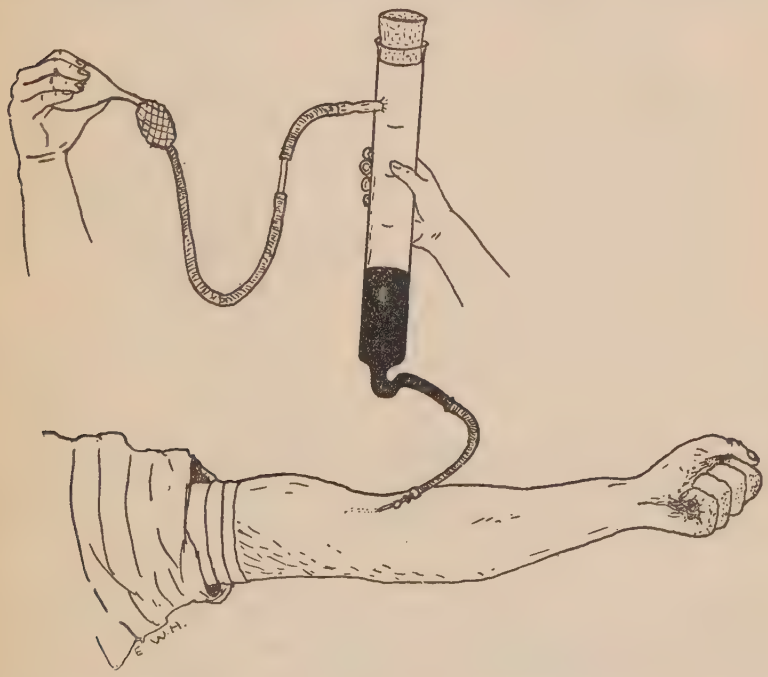


FIG. 9. THE KIMPTON-BROWN TUBE IN USE, WITH THE ADDITION OF AN ADAPTER

by means of which the blood is discouraged from clotting. The other set involves the use of syringes, in which clotting is prevented or not given time to occur.

The paraffined tube methods are largely outgrowths of Kimpton and Brown's procedure, first suggested in 1913. Kimpton and Brown devised a glass cylinder with an S-shaped cannula leading from the bottom. A cork stopper closed the top, and a perpendicular side tube emerged a little below the stopper. The apparatus is shown in figures 8 and 9.

The tubes may be obtained in various sizes, but one of a capacity of 700 cc. is best adapted for the ordinary transfusion. The success of the method depends on the complete paraffinization of the tube. This has been the chief drawback to the procedure, since the the slightest defect in the paraffine coating causes coagulation within the tube. The original method of

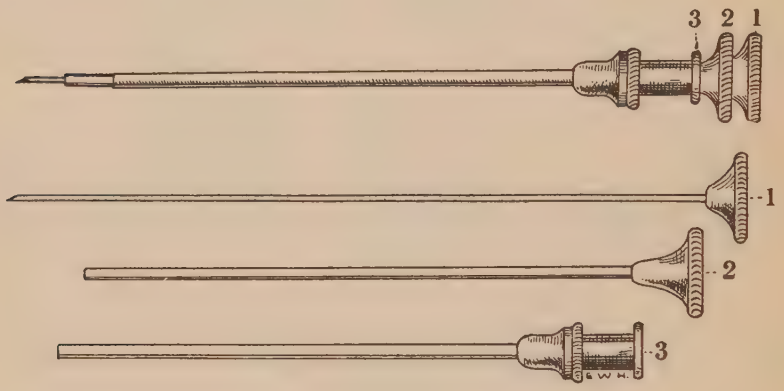


FIG. 10. LINDEMANN'S CANNULAE

Redrawn from Lindemann 1914, by permission of the *Journal of the American Medical Association.*

paraffinizing the tube was by heating and revolving it with a small piece of clean paraffine of 50°C. melting point inside. Alton (1919) suggested a better method, using a paraffin-ether solution (1:80), and coating both the tube and a rubber stopper.

Originally the tip of the glass cannula itself was inserted into the blood vessel after exposure and an incision. Later modifications involve the use of needles, eliminating the surgical approach.

Various modifications of the Kimpton-Brown tube have been suggested (Percy 1915, Kreuscher 1918, Schläpfer 1921, Moll 1926, Brooks 1926, Gabriel 1926), and one form or another of the method is still considerably used, especially in Germany (Nather 1926, Beck 1926, et al.). The method is satisfactory for the transfusion of whole unmodified blood into adults,

although in this country it has become largely superseded by syringe methods and the citrate method.

The syringe methods for the transfusion of whole unmodified blood date back to Blundell's original work in 1818 (see fig. 3). A syringe technic was successfully employed by von Ziemssen (1892), and a similar technic, the first really practical method, was described by Lindemann in 1913. This method involves six record syringes and two sets of cannulas, three to each

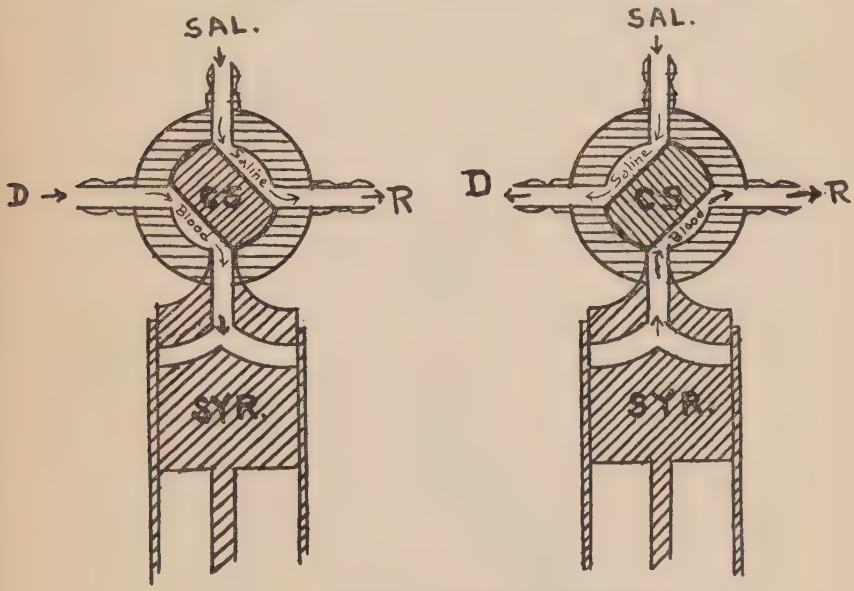


FIG. 11. UNGER'S TRANSFUSION APPARATUS

Donor's position at left, recipient's position at right. B, blood outlet; C.S., central stopper (rotates through an arc of 45 degrees); D, passage connected with donor, R, passage connected with recipient; S, passage connected with saline syringe, Syr, blood syringe, D-B, donor blood circuit; B-R, recipient blood circuit; S-R, recipient saline circuit; S-D, donor saline circuit. Redrawn from Unger 1919, by permission of the *Journal of the American Medical Association.*

set. The three cannulas telescope one within the other, the innermost one being a no. 30 gauge, ground to a fine beveled point. The outer cannula is a no. 14 gauge made to fit a Record syringe. The cannulas are shown in figure 10.

The syringes are filled with blood from the donor, the blood injected into the recipient, and the syringes washed, in rapid succession. Clotting is not given time to occur in this method. With much practice and good team

work on the part of the operators, the method is successful. Because of the skill required, it has largely been replaced by various modifications, which, as

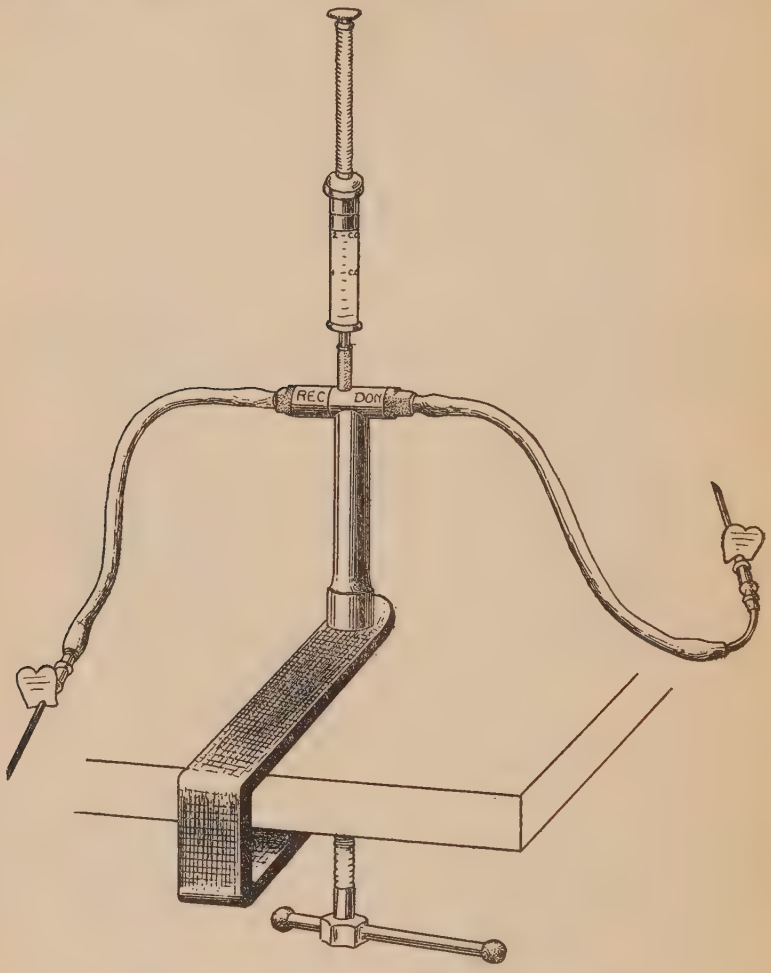
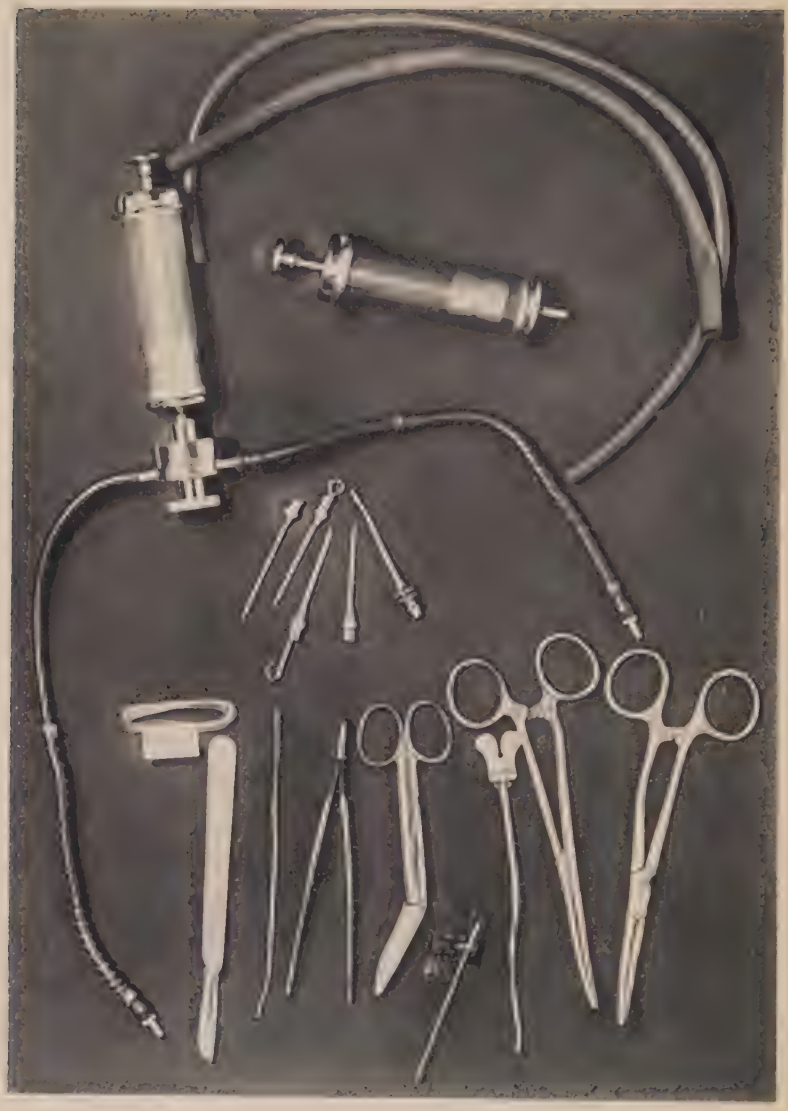


FIG. 12. THE KOSTER TRANSFUSION APPARATUS

Redrawn from Koster 1925, by permission of the *Medical Journal and Record*

in the case of the other methods, have been very numerous (Cooley and Vaughan 1913, Freund 1913, Kush 1915, Bernheim 1915, Unger 1915, Abelmann 1916, Lintz 1916, Brown 1918, Graham 1920, Dorner 1923,



FEINBLATT'S TRANSFUSION APPARATUS

From Feinblatt, *Transfusion of Blood*, copyright 1926 by the Macmillan Company, reproduced by permission.

Becart 1925, Stoll 1925, Feinblatt 1925, Koster 1925, Carleton 1926, Scannell 1926, et al.).

Unger's modification, involving a four-way stopcock, has become popular. A diagram of the apparatus is shown in figure 11. Blood is drawn into the syringe from the donor, while saline is being injected into the recipient's vein from the saline syringe by the assistant. The stopcock is then turned, and the blood in the blood syringe is injected into the recipient, while saline is being injected by the assistant into the vein of the donor. A stream of ether played on the syringe prevents clotting.

It has been claimed that this method, though generally satisfactory, does not preclude the possibility of a reflux of blood from the recipient to the donor. Donahue (1926) devised a modification of Unger's instrument in which the saline syringe is also used for blood, keeping a constant flow of whole undiluted blood from donor to recipient. Feinblatt's (1925) instrument was devised with the dual purpose of reducing the difficulties of instrumental manipulation to a minimum, and obviating the possibility of a reflux of blood from the recipient to the donor. The armamentarium for the performance of transfusion by this method is shown in plate 1. The instrument consists essentially of two disks, rotating one upon the other through an angle of 90 degrees. The proximal disk is perforated to form a chanel for communication with a Record syringe attached to it; this opening alternately becomes continuous with either of two similar openings in the peripheral disk, for the inlet and outlet, respectively. A piece of rubber tubing with an adapter is attached to the inlet, and another to the outlet. A holder which clamps on the table is provided.

A method which I have used and found satisfactory is the Koster (1925) method which likewise obviates a reflux of blood. A complete description of this method is given here, and from this the modifications in the various other technics may be readily understood. The apparatus is shown in figure 12.

The necessary apparatus is sterilized. This includes the instrument, needles, rubber tubing, two artery clamps, and two 18-inch lengths of rubber tubing for tourniquet purposes. The arms of donor and recipient are painted with iodine. A board 2 feet long is placed between the stretchers on a level with the shoulders of donor and recipient. The apparatus is screwed to the board so that it is between the elbows of the two participants. The rubber tubes are attached to the donor's and recipient's ends of the apparatus, respectively. The syringe, which delivers 2 cc. at a stroke, is mounted on the apparatus and the instrument filled with saline solution. The tourniquets being applied to the arms, the recipient's needle is introduced into a promi-

nent elbow vein. (See later in this chapter under Route and also plate 4.) The donor's needle is likewise introduced into an elbow vein of the donor. The adapters on the rubber tubes from the respective ends of the apparatus are connected to the donor's and recipient's needles, and the blood transferred by downward strokes on the syringe. When the proper amount of blood has been transferred (each syringe-full being 2 cc.) the needles are withdrawn and alcohol pads applied to the arms. The instrument should be cleaned at once.

It is important to transfer the first 10 to 30 cc. very slowly in order to carefully watch the recipient for reactions. In the case of a reaction (see Dangers at the end of this chapter) the transfusion should be stopped at once.

INDIRECT TRANSFUSION INVOLVING CITRATED BLOOD

Methods of transfusion involving anticoagulation have been practically reduced to the various modifications of the citrate method. The suggestion and first applications of sodium citrate have already been mentioned (chapter V). The credit for the earlier work goes to Hustin (1914), Agote (1915), Weil (1915), and Lewisohn (1915); and the citrate method is often referred to as Lewisohn's method. Sodium citrate is now known to be non-toxic in a dilution which is still effective as an anticoagulant. The citrate forms with the calcium in the blood a double soluble salt, rendering the calcium inert. As blood will not clot without the presence of calcium, the use of citrate effectually prevents coagulation.

There are both advantages and disadvantages to the citrate method. The disadvanages are the possible occurrence of citrate reactions, the danger that the availability and simplicity of the methods may lead to the performance of the operation without sufficient experience, and the possible bad effects of citrate upon blood. Citrate reactions are known to occur. Various workers have found reactions occurring after citrate transfusions which did not occur as frequently, if at all, after syringe transfusions (Keynes 1922, Lederer 1923, Kretzler 1924, Lewisohn 1924, Feinblatt 1926, et al.). Citrate reactions are not not usually fatal, and are generally characterized by a chill followed by a rise in temperature of 2° or 3°F. Lewisohn believes the reactions to be due to the chilling of the blood, not to the action of the citrate itself. These reactions are quite separate from those occurring as a result of incompatible blood, anaphylaxis, or the too rapid introduction of blood, which are discussed elsewhere (chapter VII).

There seems to be a variation in the tolerance of different people for sodium citrate. It has also been stated that citrate renders the corpuscles more

fragile, diminishes complement in the donor's serum, reduces the opsonic index in the plasma, reduces the phagocytic power of the leukocytes, and destroys platelets (Drinker and Brittingham 1919, Unger 1921, Colebrook and Storer 1923, Agnew 1924, Thornton 1925). However, Mellon, Hastings and Casey (1922) were not able to confirm Unger's statement concerning the destructive influence of citrate on phagocytic activity, opsonins, and complement.

The advantages of the citrate method are many, and in the minds of a large number of operators far outweigh the disadvantages. Citrate methods have simplified technic to such a point that any physician with a knowledge of intravenous therapy can perform the operation satisfactorily; they have obviated the danger of clotting; they have done away with the necessity of special apparatus; they have made possible a wide range in the time and distance of transfusion; and they have reduced to a large extent pain, excitement, and fear on the part of donor and recipient.

The apparatus required for the citrate method is shown in plate 4, figure 1. The donor's arm is prepared as previously described and a tourniquet applied. A large needle is inserted into a prominent elbow vein. The use of a large needle (10 to 14 gauge) allows the blood to flow rapidly without clotting. The blood is permitted to flow into a glass receptacle containing a 2.5 per cent solution of sodium citrate (50 cc. of the citrate solution for 450 cc. of blood). If a large vein is available it makes no difference whether the needle points towards the hand or the shoulder, but it is perhaps better in most cases to point it toward the hand. Gentle shaking of the receptacle and stirring with a sterile glass rod will mix the blood and the citrate.

The donor can help the flow of blood by slowly clasping and unclasping the hand. When the full amont of blood has been drawn, the needle is withdrawn from the vein, and an alcohol pad applied. The donor's part is then completed, but he should be kept on his back for a while.

The vessel containing the citrated blood should be placed in warm water while the recipient is being prepared. In puncturing the recipient's vein, the needle is pointed towards the head. The obviation of the danger of clotting makes possible the use of a smaller needle. The citrated blood is poured through gauze into the glass cylinder connected to the recipient's needle. A syringe with a three-way stopcock is of great value, as the operation will then require no changing of syringes. The plunger is partly withdrawn to be sure that the needle is in the vein, then the stopcock is turned, and the blood allowed to run into the recipient's vein by gravity. Warm towels may be held around the cylinder to keep the temperature of the blood up. Lewisohn has suggested the use of an especially con-

structed thermos bottle. Lindsay, Rice and Selinger (1929) have described an apparatus for maintaining an elevated temperature in citrate transfusions, in which the glass cylinder is surrounded with a metal tank into which water at the required temperature is poured. This apparatus is shown in figure 13.

FIG. 13. APPARATUS FOR CITRATE TRANSFUSION AT ELEVATED TEMPERATURE

From Lindsay, Rice, and Selinger 1929, by permission of the *Journal of the American Medical Association.*

Modifications of the citrate method have been many, but the technic as described above is still generally used. Suggested modifications have

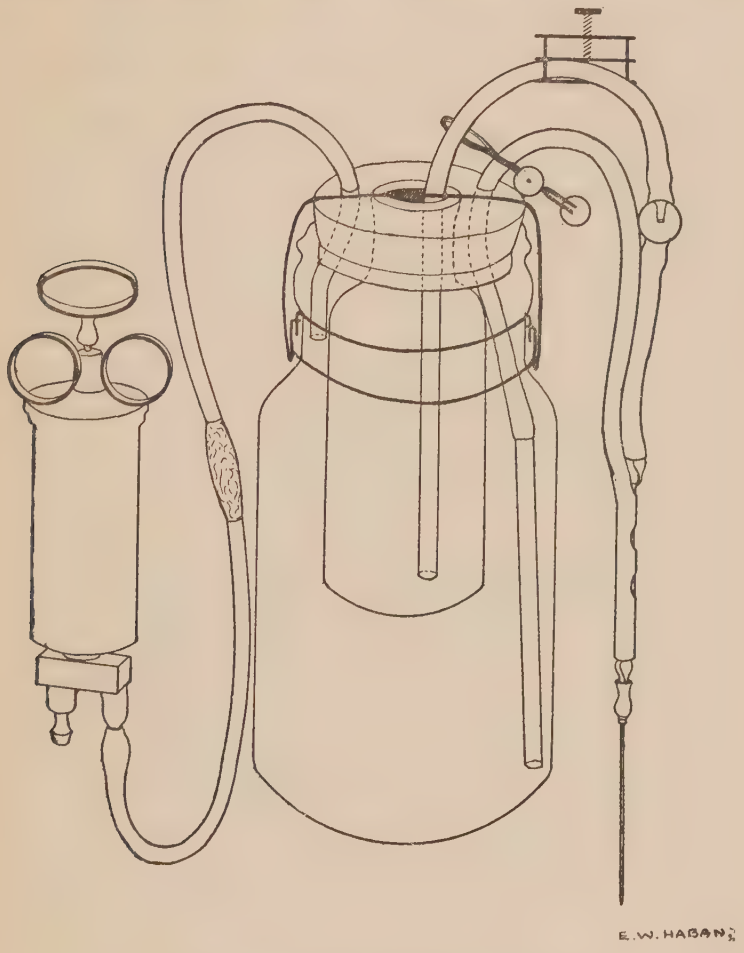


FIG. 14. HARTMAN'S TRANSFUSION APPARATUS

Redrawn from Hartman 1918, by permission of the *Journal of the American Medical Association.*

involved closed receptacles for collecting the blood, the addition of citrate under aseptic conditions, variation in the strength of the citrate, the use of force other than gravity for injecting the citrated blood, and the extension

of time between the collecting and injecting of blood. Hartman's (1922) apparatus for the citration of the blood within the donor's needle is shown in figure 14. Cowles and Antz (1923) have described a similar method. Howard's method (1915) provided for citration within the syringe. Kolmer's apparatus (1924) is representative of the closed type, and is shown in plate 2.

Rous and Turner (1916) showed the possibility of preserving erythrocytes for future transfusions. For this method, when necessary, three parts of blood are caught in a mixture containing two parts of isotonic sodium citrate solution (3.8 per cent in water). After standing, the supernatant fluid is drawn off, and the cells suspended in Locke's solution. This suspension may be kept on ice as long as a month. It must of course be warmed to body temperature before being used.

The choice of the various available methods with whole or citrated blood must be largely left to the judgment of the operator, and will be influenced most by his familiarity with a particular technic. The syringe methods, the paraffined tube methods, and the citrate methods are tried and proven, and are the technics mostly used at present.

DOSAGE

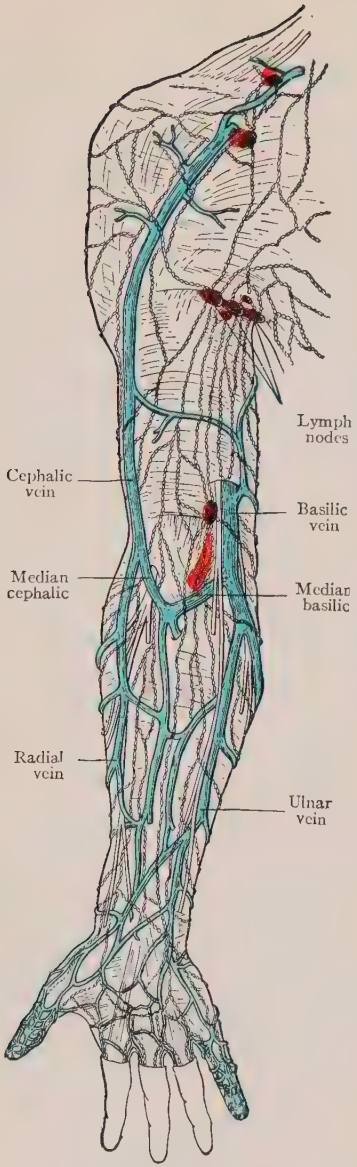
The usual dosage for adults is 500 cc., but it may vary from 300 to 750 or even 1000 cc. at the discretion of the operator. Factors influencing the amount of blood to be transfused include the age, weight, and clinical condition of the patient. Too large amounts of blood may be fatal both in children (cf. Cherry and Langrock 1916) and in adults (cf. Unger 1919). In children the dosage may vary from 50 to 150 or even 200 cc. Jones (1927) discusses the dosage for transfusion, and sets forth a standard amount of 400 cc. for the average man 5 feet 8 inches tall and weighing 150 pounds. Unger (1919), in discussing the effects of hypertransfusion, warns never to give more than 200 cc. of blood after the first cough, emphasizing that a short sharp cough is the outstanding symptom of the introduction of two much blood.

Halbertsma (1922) contributed important data on the amount of blood to be introduced into infants and children. He showed that 15 cc. of blood per kilogram of body weight would cause a rise of the red cell count of about one million per cubic millimeter of blood. This means that about 7 cc. of blood per pound of body weight would increase the red cell count by a million per cubic millimeter of blood. At this rate a 20-pound baby would receive 140 cc. of blood.

Whether to transfuse large amounts at one time or repeated doses of small

KOLMER'S APPARATUS FOR THE COLLECTION AND CITRATION OF BLOOD

From Kolmer's *Infection, Immunity, and Biologic Therapy,* reprinted by permission of the publishers, W. B. Saunders Company.



THE VEINS OF THE ARM

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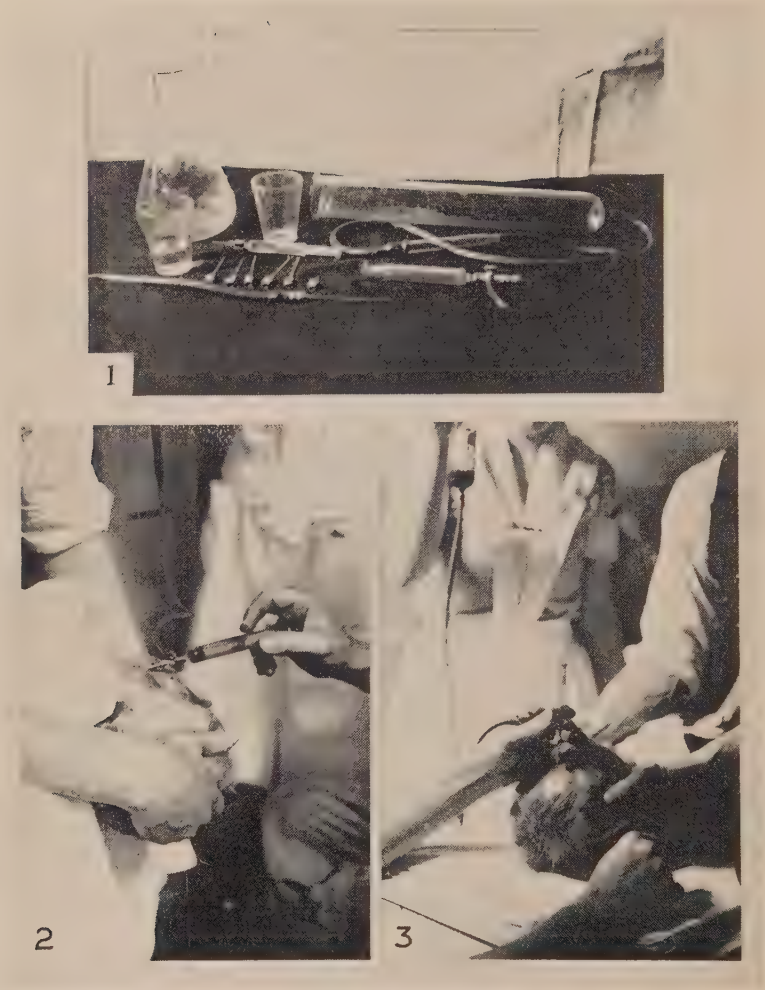

CITRATE TRANSFUSION

Fig. 1, apparatus; fig. 2, transfusion by way of jugular vein; fig. 3, transfusion by way of anterior fontanelle.

amounts in adults will depend on the disease to be treated or the urgency of the case. This subject has been treated in the chapter on indications (chapter VI).

ROUTE

The various routes which have been proposed and used will be treated separately. These include the following: intravenous, intra-arterial, into the longitudinal sinus, intraperitoneal, intramuscular, subcutaneous, per rectum, via the corpus cavernosum, and intracardiac.

The usual method of choice will be the intravenous route. For this many veins have been suggested. Where possible a prominent vein at the elbow (usually the median cephalic or the median basilic) should be used. The veins of the arm are shown in plate 3. In children, the saphenous vein at the ankle has been suggested. Falls (1923) objects to this in infants because of the inevitable post-operative soiling of the wound by urine and feces. He suggests the external jugular vein, which, located just under the platysma muscle, is easily accessible and may readily be seen when the baby cries. The head must be rotated to one side. The vein may first be exposed, but it is better to enter it without exposure if possible. Transfusion by way of the external jugular vein is shown in plate 4, figure 2.

Feinblatt (1926) prefers cutting down on a vein, when it is not available, to using some other route. Sidbury (1927) lists twenty-five transfusions which he made in infants under one year without having to cut down on the vein. In 1923, Sidbury recorded the case of a four days old infant suffering from hemorrhagic disease of the new-born, which he successfully transfused by way of the umbillical vein.

Intra-arterial transfusion is listed by Doan (1927) as a method used by Esmarch (1877) to obviate the danger of over-distension of the right side of the heart.

In young children, where it is inadvisable or impossible to enter the veins, the superior longitudinal sinus may be employed as a route for the transfusion. Until about eighteen months of age the anterior fontanelle may still be open. Transfusion by this route is shown in plate 4, figure 3. The skin in the region of the fontanelle is shaved and sterilized with iodine and alcohol. A no. 18 gauge needle with a short sharp bevel is used. It is forced through the pericranium in the mid-line, and into the sinus. When the sinus has been entered, blood will flow. The puncture is very shallow, as the sinus is directly beneath the pericranium. This method is suitable for either the citrate or the syringe technic.

The dangers of this method make its use restricted to cases where the

intravenous route is not practical. If the sinus is not in the mid-line, or if the baby's head should jerk, blood might be injected outside of the sinus.

Intraperitoneal transfusion in cases where other methods are difficult or inadvisable has been suggested by Siperstein and Sansby (1923). They showed by experiments on rabbits that the red cells of citrated blood introduced intraperitoneally are absorbed into the general circulation without harm. Siperstein (1923, 1925) reported favorable results of intraperitoneal transfusions in infants. Sansby (1925) presented evidence that erythrocytes so injected are functional. Meyer (1924), Opitz (1924), Ruh and McClelland (1923), and others, have successfully used this method. Opitz used defibrinated blood in two cases. It has been stated that blood grouping tests are unnecessary with this procedure, but this is a dangerous assumption, and compatibility tests should be made just as with any other technic.

Florey and Witts (1928) believe that this method is a rational form of treatment in the more chronic forms of anemia, although they do not think that it is likely to compare in value with the intravenous route. The blood is absorbed by the diaphragmatic lymphatics and enters the circulation by the thoracic duct. Absorption is slow, and the retention of blood for a long time in the abdominal cavity results in the danger of infection.

Cole and Montgomery (1929) obtained very satisfactory results from 117 cases of intraperitoneal transfusions. Reactions (restlessness, abdominal discomfort, etc.) occurred in less than 7 per cent of the cases. These authors warn that the intraperitoneal route should never be used if there is any question of intra-abdominal disease. While of little use in the rapid replacement of blood in shock or hemorrhage, they believe that this method possesses in every other respect all the advantages of intravenous transfusion with the additional advantages of reduced reaction and easy administration.

Subcutaneous and intramuscular transfusions are infrequently employed. Intramuscular injection in infants by way of the buttocks has already been mentioned in chapter VI. Moore and Dennis (1928) successfully used subcutaneous transfusion in children. Out of 76 patients they reported 68.5 per cent recovery. Unmodified whole blood and citrated blood seem equally effective in this method, although citrated blood is said to be absorbed more quickly. Moore and Dennis recommend 50 cc. per kilogram of body weight in new-born infants (about 23 cc. per pound), and half of that amount in older infants.

Schaefer (1918) modified the usual auto-transfusion procedure by using the blood as a rectal drip in cases of large hemorrhage in an infected field.

Shaw (1928) has suggested that in cases where veins are inaccessible in adult males, the corpora cavernosa of the penis, which are vascular lacunae,

may be used for injecting blood. Thus in emergencies there is always one "available vein" in a male patient. He lists seven successful transfusions by this route.

The direct injection of blood to the heart may be used in desperate cases (Tzanck 1922). Achard, Cournaud, and Prichet (1929) report a case of intra-cardiac injection. The patient was a woman with typhoid, in whom hemorrhages intervened. Intravenous injection was prevented by collapse of the veins. The patient was dying, and 400 cc. of citrated blood were injected into the heart, at the third intercostal space, near the left margin of the sternum. The operators state that extreme urgency is the only indication for the use of this route.

EXSANGUINATION TRANSFUSION

In recent years the use of exsanguination transfusion has come into prominence (Robertson 1924, Carlton 1926). In toxemia of severe burns, carbon monoxide poisonings, erysipelas neonatorum, and other toxemic conditions, the simultaneous withdrawal of the toxic blood from the patient and its replacement by fresh blood from a healthy donor have proven a valuable therapeutic procedure. In this method the exsanguination of the patient is begun before the transfusion. As soon as there is a weakening of the pulse, the transfusion is begun, and from then on the two operations are carried on simultaneously.

DANGERS

There are still dangers connected with the operation of transfusion, in spite of the high perfection of the various technics. Most of these have been treated in other chapters, in connection with the particular problem under discussion. They may be briefly collected and mentioned here for convenience.

Dangerous and even fatal reactions may occur when the blood of an incompatible group is introduced. This is discussed in chapter VII.

Citrate reactions may occur. These are discussed in a previous section of this chapter.

The too rapid injection of blood may cause a reaction. This is discussed in chapter VII.

The introduction of too much blood may result in a dangerous condition. This is discussed under Dosage in this chapter.

The recipient may show an anaphylactic reaction if the same donor is used several times for one recipient, or if the donor's blood contains food or

other specific proteins to which the recipient is susceptible. For this reason it is important to have the donor fast before the transfusion.

An infected donor may transmit a communicable disease to the recipient. Cases where this has happened are given in chapter VII. A reflux of blood from recipient to donor, which is theoretically possible with certain types of apparatus, may infect a healthy donor.

Multiple embolism is a rare accident occasionally recorded. Feinblatt considers it most apt to happen in the treatment of bacterial endocarditis or puerperal conditions.

Paralysis of the heart muscle brought about by strain on the heart due to transfusion has been recorded (Biesenberger 1928).

Most of the unfavorable reactions may be eliminated by careful unhurried procedure and accurate technic.

CHAPTER IX

Mendelian Inheritance

"Heredity has long been acknowledged as one of the important forces at work in causing the evident differences among men in mental and physical traits. The respect accorded by all peoples to considerations of birth, family, rank, and race provide ample evidence of this fact. The relation of heredity to the problems which have arisen in modern societies, however, has only recently been appreciated."—Sinnott and Dunn, *Principles of Genetics*,* 1925.

INTRODUCTION

There have been presented four different hypotheses concerning the mode of inheritance of the blood groups. These are, first, the hypothesis of two independent pairs of factors, conceived by von Dungern and Hirszfeld and advocated by Ottenberg and others. Second, the hypothesis of three multiple allelomorphs, suggested by Bernstein and elaborated by Snyder, Schiff, Thomsen, and others. Third, the hypothesis suggested by Furuhata and his co-workers in Japan, which, as will be pointed out in the next chapter, is essentially one of the complete linkage of two pairs of factors. Fourth, the hypothesis of partial linkage recently offered by Bauer.

In order to evaluate these various hypotheses and arrive at a logical conclusion regarding the nature of the hereditary factors underlying the blood groups, it is necessary to have in mind some fundamental facts concerning Mendelian inheritance. This chapter will be devoted to a brief discussion of some basic principles, and will be limited to such subjects as are indispensable for an adequate understanding of the heredity of the blood groups.

First of all, then, is the fundamental and practically unquestioned assumption that hereditary factors are carried on the chromosomes. The evidence for this assumption is so overwhelming that we may take it as a cardinal principle. This means that if the blood groups are inherited (and they undoubtedly are), the factors causing the appearance of the blood groups must lie somewhere on some pair or pairs of chromosomes in the cells of the human body.

Each cell of the human body (except the mature germ cells) contains in the nucleus forty-eight chromosomes: that is, twenty-four pairs. The

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mature sperms and eggs will of course each contain just half of this number. Located at various but specific places along these chromosomes are genes, or factors (probably chemical entities), which cause the development in the individual of certain definite characters.

If all the individuals of a species are alike with regard to a certain character, we can of course learn nothing as to the hereditary nature of the character. Thus, if all persons were brown-eyed, we would know nothing of the heredity of eye color. But when we find a brown-eyed person marrying a blue-eyed person, we begin to collect data on eye-color inheritance. Animals and plants, rather than man, have furnished the experimental basis for the laws of heredity, due to obvious advantages. These laws have been worked out thoroughly enough that we do not expect the discovery of new fundamental laws to be a common occurrence. A law of heredity, once worked out in any species, be it fly, corn, cow or jimson-weed, is a fundamental thing, and may be expressed in any other living form, including man. Thus, when we desire to know the hereditary nature of a human character, we do not try to work out a new law to fit it, but we first attempt to relate its mode of inheritance to some one of the fundamental laws now known.

FACTOR PAIRS (ALLELOMORPHS)

Now as to the laws which are known and understood. First of all, each hereditary character is caused to appear in an individual because of a factor or set of factors present on the chromosomes of that particular individual. Thus, there is a factor for brown eyes, which when present on its particular chromosome in the cells of an individual, causes that individual to develop brown eyes. There is likewise a factor for blue eyes, located on the same spot on the chromosome as the brown eye factor, so that a factor for blue eyes or a factor for brown eyes, but not both, can be on that chromsome at one time. Since we must study heredity by means of contrasting characters, as before mentioned, we usually think of hereditary factors going in pairs, as brown or blue eyes, feeble-mindedness or normal mind, extra thumbs or normal hands, and so on.

Chromosomes occur in homologous pairs in the cells of an individual. Thus a factor may be represented twice (once on each chromosome of the particular pair) or both members of a pair of contrasting factors may be represented in the same individual (one on one chromosome of a pair, one on the other). If both chromosomes of the pair carry the factor for brown eyes, it is easy to conceive that the person will be brown-eyed. Likewise if both chromosomes of that pair carry the factor for blue eyes, the person will be blue-eyed. If one chromosome carries the factor

for brown eyes and the other the factor for blue eyes, the person will nevertheless be brown eyed. We say that brown eyes are thus *dominant* to blue eyes. Designating B as the factor for brown eyes, and b as the factor for blue eyes, this is expressed diagrammatically in figure 15.

MULTIPLE ALLELOMORPHS

Contrasting factors occupying the same locus, or spot, on a pair of chromosomes, are known as allelomorphs. They usually occur in pairs. Sometimes, however, there is a third allelomorph in the series, or even a fourth and fifth. We are then dealing with *multiple allelomorphs*. The factor for grey eyes is a third allelomorph in the eye-color series. Only one of these

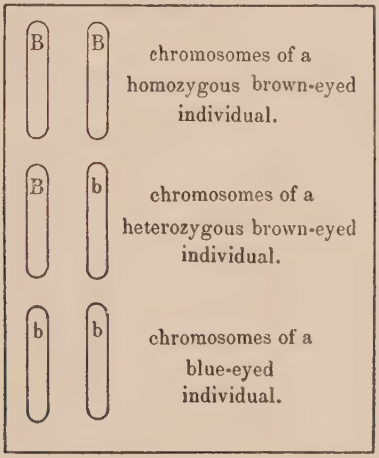

FIG. 15. ALLELOMORPHS ON THE CHROMOSOMES

three factors may be present at any one time on a single chromosome. Thus a chromosome of the pair under discussion may carry a factor for brown eyes, or one for grey eyes, or one for blue eyes. Using the same letters as before for brown and blue eyes, and designating the factor for grey eyes as b′, the possible genetic compositions of human beings in regard to eye color are shown in figure 16.

Bearing in mind that one chromosome of each pair is inherited from the father and one from the mother, the following facts are apparent from figure 16. First it may be seen that no child could be brown-eyed unless at least one of its parents was brown-eyed. Second, if both parents are blue-eyed, the offspring could be only blue-eyed. Other hereditary possibilities

may be computed from the figure. Such facts as these are at the basis of the medico-legal applications of human hereditary factors.

DIHYBRID INHERITANCE; INDEPENDENT ASSORTMENT

Thus far we have been dealing with a single pair or set of factors, that is, a pair of allelomorphs or a set of multiple allelomorphs. Now let us con-

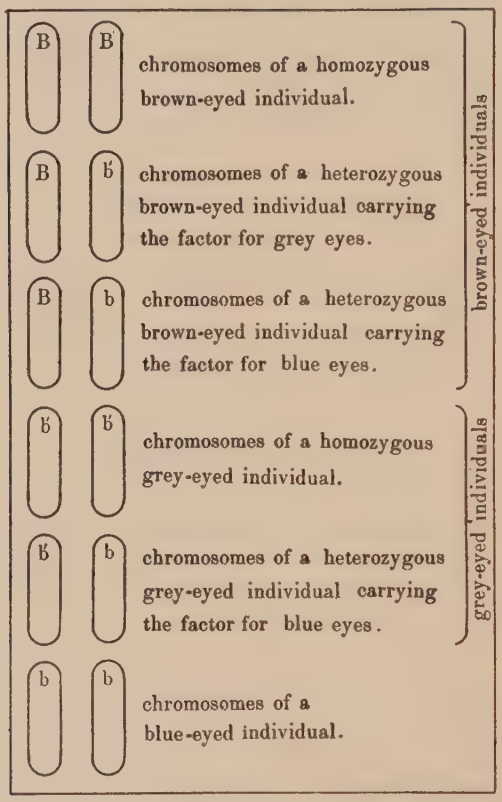
B B
chromosomes of a homozygous brown-eyed individual.
B b'
chromosomes of a heterozygous brown-eyed individual carrying the factor for grey eyes.
B b
chromosomes of a heterozygous brown-eyed individual carrying the factor for blue eyes.
brown-eyed individuals
b' b'
chromosomes of a homozygous grey-eyed individual.
b' b
chromosomes of a heterozygous grey-eyed individual carrying the factor for blue eyes.
grey-eyed individuals
b b
chromosomes of a blue-eyed individual.

FIG. 16. MULTIPLE ALLELOMORPHS ON THE CHROMOSOMES

sider the simultaneous inheritance of two different pairs of factors. It is obvious that two pairs of factors may be located either on the same chromosome, or on different chromosomes. If they are on different chromosome pairs, they will behave independently of each other. If, however, they are on the same chromosome pair, they will be transmitted together: that is, each chromosome of the pair will carry both of the factors with it wherever it goes.

Using B and b to represent brown and blue eyes, respectively, and a new pair of factors, C and c, to represent clockwise and counterclockwise direction of occipital hairwhorl, the possibilities as to the relationships of these two pairs of factors are shown in figure 17.

LINKAGE AND CROSSING-OVER

If both pairs of factors are located on the same chromosome, it is evident from figure 17 that where one factor goes, the other factor on that chromosome must go too. This condition is called linkage: that is, the two factors are linked together on the same chromosome. There is an exception to the phenomenon of linkage, however. Instead of the complete linkage of two factors on the same chromosome, it sometimes happens that two homologous

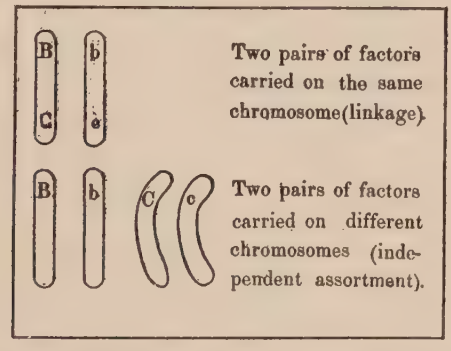

FIG. 17. FACTOR RELATIONSHIPS IN LINKAGE AND INDEPENDENT ASSORTMENT

chromosomes exchange equal parts with each other. If one of the pairs of factors is included in this exchange and the other not, the previous relations of the factors would be reversed. The farther apart on the chromosome the two factors are located, the more probable will be the reversal of relationships. If they are very close together, the exchange will affect neither or both, and no reversal of relationships will occur. The phenomenon of exchange of homologous parts of homologous chromosomes is known as "crossing-over," and is a natural phenomenon occurring at every maturation division. Crossing-over is illustrated in figure 18, top row, and the absence of crossing-over of factors due to their close proximity, even though the chromosome exchange is effected, is shown in figure 18, bottom row.

Again, when crossing-over occurs, the relationships of the factors are affected only if the individual is heterozygous for both pairs of factors: that

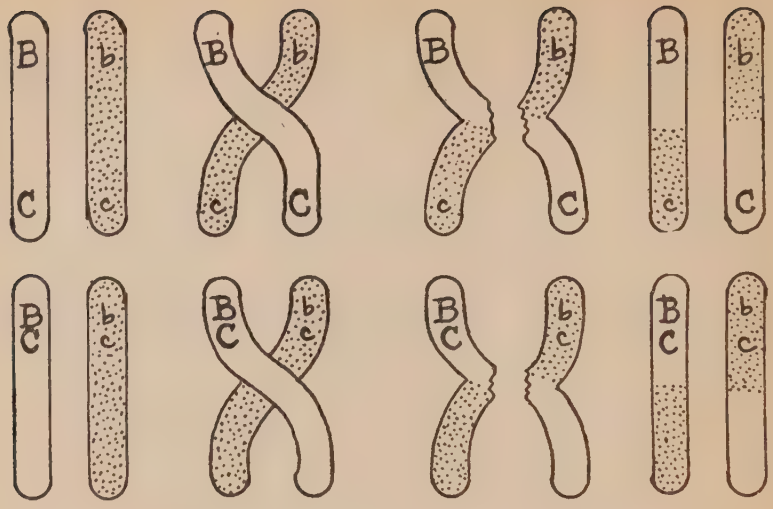

FIG. 18. LINKAGE WITH AND WITHOUT CROSSING-OVER

Top row, exchange of homologous parts of homologous chromosomes resulting in "crossing-over" with the reversal of the previous relationships of the factors. Bottom row, exchange of homologous parts of homologous chromosomes without affecting the relationships of the factors, due to their close proximity.

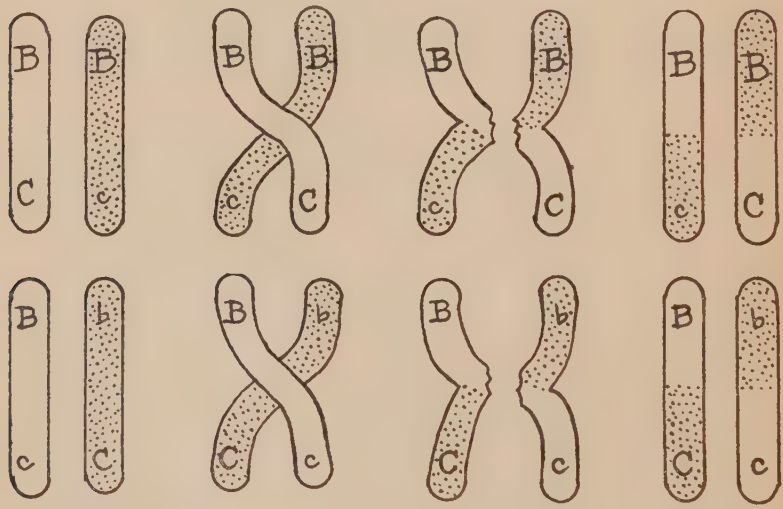

FIG. 19. CROSSING-OVER WITH AND WITHOUT DEMONSTRABLE EFFECT

Top row, crossing-over resulting in no change of relationships of the characters, and thus no demonstrable effect, due to homozygosity of one pair of factors. Bottom row, crossing-over resulting in reversal of relationships, due to heterozygosity of both factor pairs.

is, if one dominant and one recessive of each pair of factors is present. If either or both pairs are homozygous, crossing-over will not have any demonstrable effect. This is important in the linkage studies now being carried on with the blood groups. Figure 19 shows this diagrammatically.

FACTOR FREQUENCY

A common misconception concerning hereditary factors is that a dominant mutation will grow at the expense of its recessive until they occupy a numerical relationship of three to one. This is a mistake. If a dominant mutation takes place in a recessive population so that there is one dominant per thousand, there would still be at the end of a thousand years only one dominant per thousand, in the absence of selection and further mutations.

The incidence of a gene and of its allelomorph in the population and the relative occurrence of homozygous dominants, heterozygous dominants, and homozygous recessives may be calculated by simple equations. This is important in a critical analysis of blood group inheritance. Thus, in an example which I recently worked out for a series of cases on the inheritance of migraine, which proved to be a dominant character (cf. Allan 1928), the frequencies were calculated as follows:

Let M = gene for migraine
then m = gene for normal condition
And let p = frequency of M
and q = frequency of m
Then $p + q = 1$

The possible combinations of these factors are expressed by the equation $(p + q)^2 = 1$. These combinations are as follows:

	M p	m q
M p	MM p^2	Mm pq
m q	Mm pq	mm q^2

$p^2 + 2pq$ = migrainous persons
q^2 = non-migrainous persons
$q = \sqrt{\text{non-migrainous persons}}$

Since non-migrainous persons occurred in 40 per cent of the general population, the value for q may be readily obtained:

$$q = \sqrt{40} = 0.632$$
$$p = 1 - q = 0.368$$

Substituting the values of p and q in the foregoing equations, one has

p^2 = homozygous migrainous persons = 13.5 per cent MM.
$2pq$ = heterozygous migrainous persons = 46.5 per cent Mm.
q^2 = non-migranous persons = 40.0 per cent mm.

Using formulas which have been derived for the purpose, results to within 0.1 per cent of the above are obtained (13.6, 46.4) for homozygotes and heterozygotes, respectively. These formulae may be found in Johannsen (1926). However, in blood group work it is necessary to derive frequencies for multiple allelomorphic genes and polyhybrid ratios, so that the ability to derive them directly is essential. The derivations of frequencies for three multiple allelomorphs and two pairs of factors will be discussed in connection with the groups themselves in chapter X.

With this brief survey of the principles underlying the study of human heredity, we turn to the inheritance of the blood groups themselves.

CHAPTER X

The Heredity of the Blood Groups

"The iso-agglutinable elements in the red blood cells with the complementary agglutinins in the serum, probably offer the most favorable material for the study of inheritance in man. Every person belongs to one of the four blood groups, and every family is a source of data."—Ottenberg and Beres, "The Heredity of the Blood Groups," in *The Newer Knowledge of Bacteriology and Immunology*, 1928.

INTRODUCTION

Occurring as fixed biochemical entities, the four blood groups have been subject to statistical study for years. In 1908 Epstein and Ottenberg made the first suggestion that the groups might be subject to Mendelian inheritance. The groups of two families were offered as evidence: one family having both parents and four sons of group *B*, and the other family having the mother and seven sons of group *A*, the father's group in this case not being determined.

The first experimental study of this possibility was made in 1910 by von Dungern and Hirszfeld. They studied the blood groups of two generations of each of seventy-two families, comprising in all 348 individuals. From the results they concluded that the blood groups were inherited as two independent pairs of Mendelian factors, the iso-agglutinogens (A and B) being dominant to their respective iso-agglutinins (a and b). On this basis, a specific agglutinogen could not appear in a child unless it was present in at least one of the parents. This last fundamental observation has never been seriously questioned to the present day, although the assumption of two independent pairs of factors to explain it seems no longer warranted.

Von Dungern and Hirszfeld's conclusions were confirmed and upheld by many later workers in all parts of the world (Learmonth 1920, Weszeczky 1920, Ottenberg 1921, 1922, 1923, Keynes 1922, Tebbutt and McConnel 1922, Jervell 1923, Dyke and Budge 1923, Kirihara 1924, Plüss 1924, Mino 1924, Hirszfeld, Hirszfeld and Brokman 1924, Dossena 1924, Avdieva and Grizevicz 1925, Furuichi 1925, Staquet 1926).

Buchanan (1922, 1923) was the only writer to take exception to the general conclusions, and especially to their applications to medico-legal problems. He based his objections on several families studied by him

which gave results not in accord with the Mendelian expectation. The weaknesses in Buchanan's arguments have been many times pointed out (Ottenberg, 1922, 1923; Keynes, 1922; Gichner, 1922; Snyder, 1924–1927, et al.), and his objections have never been taken seriously.

The medico-legal application, which is discussed in the next chapter, is based on the simple fact, now well established, that an agglutinogen never appears in a child unless it was present in at least one of the parents. The cases in which an agglutinogen not present in one of the parents has appeared in a child (Learmonth 1920, Buchanan 1923, Mino 1924, Landsteiner and Levine 1928, Snyder, unpublished data) present three possibilities: (1) the Mendelian laws do not apply in a simple way to the blood groups; (2) the alleged parentage is incorrect; (3) there has been a mistake in observation or technic. Such exceptions are sufficiently rare that they do not invalidate the assumption that the groups are subject to simple Mendelian inheritance. This leaves two explanations for such anomalous cases: illegitimacy or mistakes in technic. The smaller number of exceptions occurring in the later work indicates that many incompatible results of the earlier workers were due to faulty technic. Most of the later workers have attributed their anomalous cases to illegitimacy, and in several cases there has been evidence apart from the blood groups of the illegitimacy.

One fundamental law, then may be taken as established. Isohemagglutinogens do not appear in a child unless they were present in at least one of the parents. Conversely, when neither parent has an agglutinogen, none of the children will have it. Von Dungern and Hirszfeld's assumption of two independent pairs of factors to explain this, however, is being seriously questioned after fifteen years of acceptance, and in fact, is no longer tenable.

In 1924, I began a series of studies on the inheritance of the groups. As data accumulated, it was evident that the fundamental assumption outlined above was correct, but it soon became apparent that in certain unions all the expectations were not being realized. For example, in unions of groups *O* and *AB*, only children of groups *A* and *B* were being obtained. While some of my early data was being puzzled over, a paper appeared by Bernstein (1925), suggesting a hypothesis of three multiple allelomorphs, based on mass statistics, and without giving any original family histories. It was immediately apparent to me that my family histories fitted this hypothesis perfectly, and furnished critical tests of the two hypotheses. I continued the collection of family blood group data until 200 families were available, then published results confirming and upholding Bernstein's hypothesis (Snyder 1926). A summary of these results is given in table 8. Since then

I have accumulated several hundred additional family histories, which are being published in connection with linkage studies.

Other workers have upheld this hypothesis (Schiff 1927, Streng and Ryti 1927, Sievers 1927, Preger 1927, Lattes 1927, Thomsen 1927, 1928, et al.), while some have not as yet fully accepted it (Hirszfeld 1926, Ottenberg and Beres 1928).

Early in 1927 personal correspondence with Dr. Furuhata of Kanazawa, Japan, disclosed the fact that he was working along similar lines, and had published in 1925, in Japanese, a hypothesis somewhat similar to Bernstein's, but differing in that it considered factors in the serum as well as those in the cells, thus being in effect one of complete linkage of two pairs of

TABLE 8

INHERITANCE OF THE BLOOD GROUPS OF 200 FAMILIES STUDIED BY THE AUTHOR

MATING	NUMBER OF FAMILIES	CHILDREN IN GROUP			
		O	*A*	*B*	*AB*
O × *O*	44	151	—	—	—
O × *A*	73	88	176	—	—
A × *A*	32	15	88	—	—
O × *B*	22	25	—	41	—
B × *B*	3	—	—	12	—
A × *B*	17	9	15	14	23
O × *AB*	5	—	10	13	—
A × *AB*	3	—	6	2	4
B × *AB*	1	—	—	2	1

factors. Within the last year this hypothesis has appeared in English (Furuhata and Kishi 1927, Furuhata 1928).

There were in the literature, however, a good many cases incompatible with Bernstein's and Furuhata's hypotheses. To reconcile these, Bauer (1928) proposed a hypothesis involving two pairs of factors linked on the same pair of chromosomes, with a certain amount of crossing over between them. There are thus at present four separate hypotheses of the inheritance of the blood groups. These will be compared and discussed in some detail.

Von Dungern and Hirszfeld's original hypothesis of two independent pairs of factors was the logical assumption to make from their data. It is only as later and more extensive heredity results have been accumulated that exceptions requiring a modified hypothesis have been noted. The gathering of data on the racial distribution of the four groups also offered unique

material with which to test the soundness of the older hypothesis. This was the test which Bernstein used, and upon which he based his conclusions, which have since been confirmed by actual family data.

A large number of peoples have been studied for their blood groups, and the proportions of the four groups are known for many nationalities. The anthropological bearing of these results will be discussed in chapter XIV, but their importance as a test of the mode of inheritance of the groups will be outlined here.

THE STATISTICAL BASIS FOR THE TRIPLE ALLELOMORPH HYPOTHESIS

It has been amply established that the agglutinogens are usually present at birth, but that the agglutinins may be delayed in their appearance for several months (see chapter IV). Tebbutt and McConnel (1922), noting this, have suggested that the agglutinogens probably preceded likewise in

TABLE 9

GENETIC FORMULAS OF THE FOUR BLOOD GROUPS

GROUP	TWO INDEPENDENT PAIRS OF FACTORS	THREE MULTIPLE ALLELOMORPHS
O	aabb	OO
A	AAbb, Aabb	AA, AO
B	aaBB, aaBb	BB, BO
AB	AABB, AABb AaBB, AaBb	AB

evolution. This assumption, however, is not warranted by the fact that primitive or long-isolated peoples show a very high percentage of group *O* (lacking agglutinogens). It will be shown in chapter XIV that primitive man was probably all of group *O*, the occurrence of the agglutinogens A and B being due to later independent mutations. The fact that agglutinogens have been found in the anthropoid apes need not invalidate this assumption, as will be pointed out in chapter XIV.

If, then, A and B are separate mutations, group *AB* must have originated by the union of groups *A* and *B*. Knowing the proportions of groups *A* and *B* in any race, the proportion of group *AB* can be calculated. This may be done independently for the two-factor-pair hypothesis and for the triple-allelomorph hypothesis. The genetic formulas of the four groups on these hypotheses are given in table 9.

On the basis of the hypothesis of two independent pairs of factors, A, a, and B, b, the calculation of group *AB* would be as follows:

Let p = frequency of A
q = frequency of a
r = frequency of B
s = frequency of b
Then $p + q = 1$, $r + s = 1$

The possible combinations of these factors are expressed by the equation $(p + q)^2 \cdot (r + s)^2 = 1$. From this the following combinations are formed:

	AA p^2	2Aa $2pq$	aa q^2
BB r^2	AABB p^2r^2	2AaBB $2pqr^2$	aaBB q^2r^2
2Bb $2rs$	2AABb $2p^2rs$	4AaBb $4pqrs$	2aaBb $2q^2rs$
bb s^2	AAbb p^2s^2	2Aabb $2pqs^2$	aabb q^2s^2

From this the proportions of the four groups are reckoned as follows:

Group O $= q^2s^2$
Group A $= p^2s^2 + 2pqs^2 = s^2(1 - q^2)$
Group B $= q^2r^2 + 2q^2rs = q^2(1 - s^2)$
Group AB $= p^2r^2 + 2pqr^2 + 2p^2rs + 4pqrs = (1 - q^2)(1 - s^2)$

From the first three above, s^2 and q^2 are calculated.

$$s^2 = \frac{O}{O + B}$$

$$q^2 = \frac{O}{O + A}$$

When the percentage of group AB in any people is calculated on this basis, it does not agree with the percentage actually found, but is always much higher than the observed percentage. For example, the calculated percentages of group AB in English and Greeks are 6.5 and 15.5, respectively. The observed percentages are only 3.0 and 4.0. These discrepancies hold throughout the races studied.

If we calculate the proportion of group *AB* in various races on the basis of triple allelomorphs, A, B, and O, however, they agree more accurately with the observed percentage. This is done as follows:

Let p = frequency of A
q = frequency of B
r = frequency of O
Then $p + q + r = 1$

The possible combinations of these factors are expressed as $(p + q + r)^2$. In this way the following combinations are formed:

	A p	B q	O r
A p	AA p^2	AB pq	AO pr
B q	AB pq	BB q^2	BO qr
O r	AO pr	BO qr	OO r^2

From this the proportions of the four groups are reckoned as follows:

Group *O* $= r^2$
Group *A* $= p^2 + 2pr$
Group *B* $= q^2 + 2qr$
Group *AB* $= 2pq$

From this, p, q, and r are calculated.

$$\text{group } O + \text{group A} = r^2 + 2pr + p^2 = (r + p)^2$$
$$r + p = \sqrt{\text{group } O + \text{group } A}$$
$$p = \sqrt{\text{group } O + \text{group } A} - r$$
$$p = \sqrt{\text{group } O + \text{group } A} - (1 - p - q)$$
$$q = 1 - \sqrt{\text{group } O + \text{group } A}$$

Similarly,

$$p = 1 - \sqrt{\text{group } O + \text{group } B}$$
$$r = \sqrt{\text{group } O}$$

When we reckon the percentage of group *AB* on this basis, the agreement is very close. Using the same examples, the calculated percentages for English and Greeks are 2.8 and 5.6, respectively, agreeing closely with the observed percentages of 3.0 and 4.0. Likewise the equation $p + q + r = 1$ is in each race very accurately fulfilled.

On the above considerations Bernstein based his hypothesis of multiple allelomorphs. The mathematical methods he used have been criticized by Mendes-Correa (1927) and Ottenberg and Beres (1928). Mendes-Correa contends that Bernstein's mathematical assumptions may be reduced to an identity, and are therefore untenable. Mendes-Correa is simply playing with figures, and works in a circle. Moreover, as Landsteiner and Levine (1928) have pointed out, his objections would imply that the formula $p + q + r = 1$, holds for arbitrarily chosen values, which is obviously not the case. Ottenberg and Beres contend that the value of r may be derived by other formulas than those used above, and that when this is done, widely varying values of r are obtained. Bernstein's formulas are more accurate, however, basing the value of r purely on the observed proportions of the groups, and not on the derived frequencies of the other factors.

THE HEREDITARY BASIS FOR THE TRIPLE ALLELOMORPH HYPOTHESIS

The hypothesis of triple allelomorphs may have been originally based only on mass statistics, but its present validity rests on observed hereditary data. The later inheritance results of workers in all parts of the world uphold almost unconditionally this view.

It will be noted that in order to conform with the new designations of the groups, the recessive factor formerly known as R is here designated as O. Since the dominant groups *A* and *B* may carry the recessive O, it is proper from a genetic standpoint to speak of the groups in terms of their phenotypes, as *O*, *A*, *B*, and *AB*, thus bringing genetic and serologic terminology into line.

The inheritance results to be expected on the two hypotheses under consideration are the same except where a parent of group *AB* is involved. A comparison of the two hypotheses in this respect is given in table 10.

It will be seen from table 10 that the offspring of matings involving a parent of group *AB* constitute critical tests of the two hypotheses. Crosses of groups *O* and *AB* are especially good tests, since the offspring should be only of groups *A* and *B* on the basis of multiple allelomorphs, but of all four groups on the basis of independent pairs of factors. Table 11 summarizes all of the unions of groups *O* and *AB* recorded to date.

On the hypothesis of three multiple allelomorphs, such unions should

result in offspring of the proportion of 50 per cent group *A* and 50 per cent group *B*. On the basis of independent pairs of factors, the offspring would be of all four groups, and the percent in each group would vary with the naturally occurring proportions of the groups in the particular race being studied. In general, however, there would always be expected more of group *AB* than of any other group. It is a simple matter, when the frequencies of the hereditary factors in a particular race are known, to calculate the expected proportions of the four groups in the offspring of the various matings for that race (see chapter IX). Streng (1927) has calculated and published generalized formulas for the expectation of the percent of each group in the offspring of the various possible matings for any race. Such

TABLE 10

THEORETICAL RESULTS OF BLOOD GROUP CROSSES

MATING	INDEPENDENT PAIRS	TRIPLE ALLELOMORPHS
$O \times O$	O	O
$O \times A$	O, A	O, A
$A \times A$	O, A	O, A
$O \times B$	O, B	O, B
$B \times B$	O, B	O, B
$A \times B$	O, A, B, AB	O, A, B, AB
$O \times AB$	O, A, B, AB	A, B
$A \times AB$	O, A, B, AB	A, B, AB
$B \times AB$	O, A, B, AB	A, B, AB
$AB \times AB$	O, A, B, AB	A, B, AB

proportions are of course dependent on the frequencies of the hereditary factors in the particular race under discussion.

It is easy to see that in table 11 the results favor the hypothesis of multiple allelomorphs. There are however, certain small proportions of groups *AB* and *O* among the offspring. How shall these be explained? In the first place, it will be observed that the larger number of these exceptions occurred in the earlier work (before 1925). It is only since then that especial emphasis has been placed on careful technic, precautions against pseudo-agglutination, tests for titer, and so on. It may well be that some of the earlier exceptions were due to lack of sufficient familiarity with the technic. In the second place, several of the recent workers have definitely attributed their anomalous cases to known illegitimacy (Thomsen 1927, Furuhata 1927, Mazza and Iraeta 1928). The child of group *O* in one of my own *O* and *AB* families (mother *O*, alleged father *AB*), is known to be illegitimate.

Kliewe and Nagel (1927) published some hereditary results in which there were several exceptions to the hypothesis of triple allelomorphs which could not be explained on the basis of illegitimacy. For example, they reported two group *O* children of a group *AB* mother, a result which could not occur on this hypothesis regardless of the group of the father. Kliewe (1928)

TABLE 11

RECORDED UNIONS OF GROUPS *O* AND *AB*

INVESTIGATOR	NUMBER OF FAMILIES	CHILDREN IN GROUP			
		O	*A*	*B*	*AB*
Von Dungern and Hirszfeld, 1910	4	2	2	2	3
Learmonth, 1920	3	0	0	0	12
Avdeieva and Grizevicz, 1921	2	2	0	0	1
Keynes, 1922	1	0	0	2	1
Tebbutt and McConnel, 1922	2	0	7	5	0
Ottenberg, 1922	2	0	5	5	0
Oyanada, 1922	3	0	6	0	0
Buchanan, 1923	4	6	2	2	1
Jervell, 1923	3	0	5	2	0
Dyke and Budge, 1923	1	0	0	1	0
Kirihara, 1924	6	1	9	8	0
Plüss, 1924	7	2	8	8	1
Mino, 1924	4	0	5	4	0
Hirszfeld, Hirszfeld, and Brokman, 1924	1	0	2	0	0
Dossena, 1924	7	2	2	3	0
Furuichi, 1925	9	2	7	7	2
Kawaishi and Furuhashi, 1925	4	2	11	8	2
Staquet, 1925	2	8	9	2	1
Sievers, 1927	14	0	30	29	0
Landsteiner and Levine, 1928	6	2	10	11	0
Thomsen, 1928	43	0	50	60	1*
Furuhata, 1928	50	1*	61	57	0
Vuori, 1929	21	0	36	42	0
Snyder, to date	15	1*	31	35	0

* Known to be illegitimate.

later published another paper retracting these results, explaining them by having confused two unrelated families of the same name.

Results such as these account for some of the anomalous cases. If it is argued that the finding of children of groups *O* and *AB* by some investigators is evidence against the hypothesis of multiple allelomorphs, it must be

pointed out that the total absence of children of group *O* and especially of group *AB* in the results of other investigators is even stronger evidence against the hypothesis of independent pairs of factors.

That exceptions to the general expectations on any hypothesis do occur is attested by families involving matings of *O* and *O*. In this case neither parent has any agglutinogen, and the children should therefore all lack them;

TABLE 12

RECORDED UNIONS OF GROUPS *O* AND *O*

INVESTIGATOR	NUMBER OF FAMILIES	CHILDRN IN GROUP *O*	CHILDREN IN OTHER GROUPS
Von Dungern and Hirszfeld, 1910	11	25	—
Learmonth, 1920	9	18	1 in group *A*
Keynes, 1922	2	4	—
Tebbutt and McConnel, 1922	5	17	—
Ottenberg, 1922	12	25	—
Buchanan, 1923	8	17	3 in group *AB* 2 in group *B* 8 in group *A*
Jervell, 1923	2	5	—
Dyke and Budge, 1923	30	30	—
Kirihara, 1924	6	20	—
Plüss, 1924	12	27	—
Mino, 1924	12	31	5 in group *A*
Hirszfeld, Hirszfeld, and Brokman, 1924	7	19	—
Dossena, 1924	31	31	—
Avdeieva and Grizevicz, 1921	16	33	—
Staquet, 1926	9	35	1 in group *A*
Thomsen, 1927	41	126	1 in group *A*
Sievers, 1927	30	101	—
Kliewe, 1928	5	10	—
Landsteiner and Levine, 1928	34	140	3 in group *A* 1 in group *B*
Furuhata, 1928	105	216	—
Vuori, 1929	53	154	1 in group *B*
Snyder, to date	131	516	1 in group *B*

that is, the children should all be of group *O*. In table 12 are summarized all the unions of groups *O* and *O* thus far recorded. This table shows likewise some exceptions. These exceptions are not only to the triple allelomorph hypothesis, but to any Mendelian explanation. They are undoubtedly due to mistakes in technic or to illegitimacy. This type of mating offers the most striking example of the fundamental fact that an

agglutinogen never appears in a child unless it is present in one or both of the parents.

The exceptions to the multiple allelomorph hypothesis are more numerous than the exceptions to a general Mendelian explanation. When only the work of the last three or four years is considered, however, this proportion becomes more even. The later work is presenting overwhelming evidence in favor of the hypothesis of multiple allelomorphs.

THE HYPOTHESIS OF COMPLETE LINKAGE

In Japan, Furuhata and his co-workers have evolved a hypothesis similar to Bernstein's, but differing from it in that it involves hereditary factors for the agglutinins in the cells as well as for the agglutinogens in the serum. Furuhata speaks of his hypothesis as one of triple allelomorphs, but a little study suffices to show that he is really dealing with two pairs of factors, A and a, B and b, differing from von Dungern and Hirszfeld's similar factors in that they are linked on a single pair of chromosomes. Furuhata assumes these factors to lie so close together that they are completely linked: that is, no crossing over occurs between them. He further assumes that A is always linked with b, and B with a, never A with B. This assumption is unwarranted, as we must suppose the possibility of a mutation taking place on a chromosome on which another gene has already mutated.

TABLE 13

GENETIC FORMULAS OF THE FOUR BLOOD GROUPS ON THE HYPOTHESIS OF COMPLETE LINKAGE

GROUP	FORMULA
O	(ab) (ab)
A	(Ab) (Ab), (Ab) (ab)
B	(aB) (aB), (aB) (ab)
AB	(Ab) (aB)

The genetic formulas of the four groups on this hypothesis are shown in table 13.

It will be seen that if Furuhata's assumptions are correct, the results of his hypothesis will be exactly the same as those of Bernstein's. The hypotheses differ only in serologic bases and minor genetic implications. A plausible serologic explanation for the triple allelomorph hypothesis is outlined in chapter II. From a genetic standpoint, multiple allelomorphs are to be preferred to complete linkage.

THE HYPOTHESIS OF PARTIAL LINKAGE

To explain the occasional appearance of children of groups *O* and *AB* in matings of *O* and *AB*, Bauer (1928) postulated the presence of two pairs of

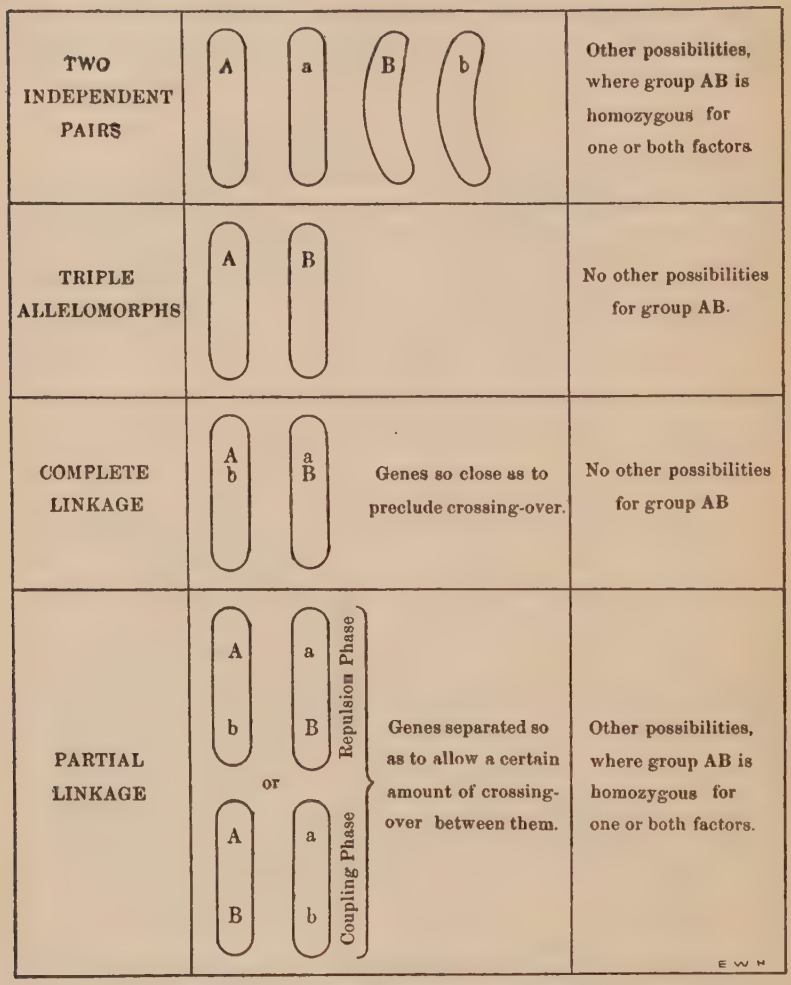

FIG. 20. CHROMOSOME COMPOSITIONS OF A DOUBLE HETEROZYGOTE OF GROUP *AB* ON THE VARIOUS HYPOTHESES OF BLOOD GROUP INHERITANCE

genes A and a, B and b, not independent, like those of von Dungern and Hirszfeld, and not completely linked, like those of Furuhata, but located on the same pair of chromosomes far enough apart to allow crossing over

between them. In other words, the factors are assumed to be partially linked. It will be seen by referring back to chapter IX that the crossing over would only affect the relationships of the genes when it took place in an individual of group *AB* of the formula (Ab) (aB). By assuming 11 per cent crossing over, Bauer claims that results would be expected equivalent to those actually obtained. On this hypothesis it must be assumed that the combinations (Ab) or (aB) are more numerous than the combination (AB).

Bauer loses sight of the fundamental genetic fact, however, that after a long period of time the numerical dominance of the combinations (Ab) and (aB) over (AB) would be reduced, so that now after hundreds of years, the four combinations (ab), (Ab), (aB), and (AB) would be found in the germ cells of group *AB* individuals in proportions comparable to those occurring in independent assortment. Thus when studied by mass family histories, this hypothesis would be identical in results with that of von Dungern and Hirszfeld, and subject to the same objections. When the individual family histories were scrutinized on this hypothesis, certain *O*-and-*AB* families should give mostly *A* and *B* children, and an equal number of families should show mostly *O* and *AB* children. In addition, some families with the parents of groups *O* and *AB* should produce only *A* and *AB* offspring, while others should produce only *B* and *AB* offspring. Thus the individual family histories also fail to bear out this hypothesis.

In order to clearly visualize the genetic implications of the four hypotheses, figure 20 is presented, showing the chromosome complex of a double heterozygote individual of group *AB* for each hypothesis.

Nearly four thousand families, involving more than fifteen thousand individuals, have been studied and recorded by various workers to date. The results do not point to any one of the four hypotheses with absolute certainty, but they strongly favor that of triple allelomorphs. More families must be studied under carefully controlled conditions, including careful checks for titer, precautions against pseudo-agglutination, and other aberrant phenomena. As Doan puts it, "We are now in the period of the accumulation of hypothesis-testing data, with its necessary increment of contemporary doubting, modification and disagreement." The objections to three of the hypotheses, however, together with the confirmation of the multiple allelomorph hypothesis afforded by later workers, are making it necessary for us to accept this hypothesis and reject the others.

CHAPTER XI

The Blood Groups in Legal Medicine

"Praktische Erfolge sind genau in den Grenzen der theoretischen Voraussage erzielt worden. Enttäuscht sein kann nur, wer Uebertriebenes erwartet hat. Eine *allgemeine* Lösung des Problems der Bestimmung der Vaterschaft war von der Landsteinerschen Reaktion nicht zu erhoffen. Die Bedeutung der Blutuntersuchung für die Lösung dieses uralten Problems liegt aber nicht nur in dem, was bis jetzt erreicht wurde. Ebenso wichtig scheint es mir, dass die bisherigen Erfolge zu einer *systematischer* Heranziehung *anderer* erblicher Merkmale anregen. Hier Richtung und Ansporn zu geben, ist eine dankbare Aufgabe der Blutgruppenforschung."—Schiff, *Die Blutgruppen und ihre Anwendung vor Gericht*, 1927.

When once the hereditary behavior of a pair of human characters is understood, it is entirely possible to make a practical application of this knowledge in cases of disputed parentage. By referring back to chapter IX, it will be seen that a dominant character appearing in an individual (animal or plant), is evidence that at least one of the parents showed that dominant character. The appearance of a recessive character in an individual does not imply that one of the parents showed the character, as it might have been carried hidden in the germ cells, only to reappear when it was received in double dose (that is from both the sperm and the egg) by the offspring.

The hereditary factors for human isohemagglutinogens have been amply demonstrated to be dominant factors (chapter X). This means that when an agglutinogen is present in a child, at least one of its parents must have had it. This becomes of practical application in cases of disputed parentage. For example, if a child shows the presence of agglutinogen A, and the mother does not, the father must have possessed this agglutinogen. If the paternity of the child lies between two men, one of whom shows agglutinogen A and the other not, the man having the agglutinogen must be the father. Of course if both men possess agglutinogen A, (that is, are of group *A* or *AB*), nothing could be said as to the paternity on this basis. Either man could be the father as far as the evidence from the blood groups is concerned.

Only in certain combinations of the groups of mother and child is it possible to restrict the group of the father. In certain cases the father could be of any of the four groups. In certain other combinations, however,

TABLE 14

PREDICTION OF REMAINING PARENT GROUPS*

KNOWN CHILDREN IN GROUP	ONE PARENT KNOWN TO BE IN GROUP	OTHER PARENT MUST BE IN GROUP
O	O	O, A, or B
O	A	O, A, or B
O	B	O, A, or B
O	AB	†
A	O	A or AB
A	B	A or AB
B	O	B or AB
B	A	B or AB
AB	O	‡
AB	A	B or AB
AB	B	A or AB
AB	AB	A, B, or AB
O and A	O	A
O and A	A	O, A, or B
O and A	B	A
O and A	AB	†
O and B	O	B
O and B	A	B
O and B	B	O, A, or B
O and B	AB	†
O and AB	O	‡
O and AB	A	B
O and AB	B	A
O and AB	AB	†
A and B	O	AB
A and B	A	B or AB
A and B	B	A or AB
A and AB	O	‡
A and AB	A	B or AB
A and AB	B	A or AB
A and AB	AB	A, B, or AB

* In the combinations which are not given in this table, the other parent could belong to any of the four groups.

† Impossible; neither parent could be of group *AB*, on the hypothesis of multiple allelomorphs.

‡ Impossible; neither parent could be of group *O*, on the hypothesis of multiple-allelomorphs.

TABLE 14—*Concluded*

KNOWN CHILDREN IN GROUP	ONE PARENT KNOWN TO BE IN GROUP	OTHER PARENT MUST BE IN GROUP
B and AB	O	‡
B and AB	A	B or AB
B and AB	B	A or AB
B and AB	AB	A, B, or AB
O, A, and B	O	‡
O, A, and B	A	B
O, A, and B	B	A
O, A, and B	AB	†
O, A, and AB	O	‡
O, A, and AB	A	B
O, A, and AB	B	A
O, A, and AB	AB	†
O, B, and AB	O	‡
O, B, and AB	A	B
O, B, and AB	B	A
O, B, and AB	AB	†
A, B, and AB	O	‡
A, B, and AB	A	B or AB
A, B, and AB	B	A or AB
A, B, and AB	AB	A, B, or AB
O, A, B, and AB	O	‡
O, A, B, and AB	A	B
O, A, B, and AB	B	A
O, A, B, and AB	AB	†

the group of the father can be predicted accurately. This prediction of the group of one parent from a knowledge of the groups of the child and of the other parent is slightly different on the various hypotheses of inheritance discussed in the preceding chapter.

In 1921 and 1922, Ottenberg presented tables showing the various possibilities of determining parentage by the blood groups. These tables were based on von Dungern and Hirszfeld's hypothesis of two independent pairs of factors. The adoption of the newer hypothesis of heredity modifies to a certain extent these tables. None of the earlier tests are invalidated. On the contrary, the application becomes even more strict. The possibilities of the group of the remaining parent are limited in the cases where

a known parent of group *AB* is involved. The medico-legal applications on the newer hypothesis have been presented in my previous articles (1927). Table 14 summarizes all of the cases where the group of the remaining parent may be predicted.

In practice, it will seldom be expedient to use the parts of the table involving two or more children. Each child's parentage must generally be considered as presenting an individual problem, and must be separately studied. The complete list of possibilities, however, is given in the table for use in those cases where more than one child is known to be the offspring of a single father.

As long as any question remains as to the efficiency of the technic of grouping or the accuracy of the hypothesis of inheritance, the use of the medico-legal applications of the blood groups must be attended with extreme caution. With proper technic, however, the tests are valuable. And all those cases which do not involve a known parent of group *AB* or a child of group *AB* are valid on any inheritance hypothesis. The cases involving group *AB* are modified by the new hypothesis. I am myself convinced that the triple allelomorph hypothesis is amply proven, and that all of the medico-legal applications as given in table 14 are accurate and applicable.

In the last one hundred families which I have studied for blood group inheritance, I have tried out the medico-legal application as a sort of test. In each case I determined the blood groups of the mother and children, then predicted the group of the father before grouping his blood. In those cases in which a prediction was possible, I made the prediction correctly in all except one case. This was a case where a mother and child were group *O*, and the alleged father group *AB*. Careful inquiry revealed the fact that the child was illegitimate.

Other hereditary characters are of similar importance in determining disputed parentage. It must not be thought that the blood group factors have any mysterious potency in such cases by virtue of being hidden in the blood. They have an especially extensive application, it is true, because they are based on a series of three allelomorphs in which the heterozygous double dominant is phenotypically recognizable. But any character in which the dominant and recessive can be clearly distinguished, and the hereditary behavior of which is accurately known, may have similar medico-legal application. Thus in the case of eye color, where brown is dominant to blue, a brown-eyed child of a blue-eyed mother must have a brown-eyed father. Other applications may be worked out for eye color. As an example of the potential danger where the dominant character can not always be clearly recognized, however, may be cited the fact that among the thou-

sands of cases of eye color inheritance which have been studied, a very occasional instance is noted where a person genetically brown-eyed has the brown pigment restricted to a few brown flecks, so that the eye at first glance looks blue. Such cases are extremely rare, and could be avoided entirely by a proper technic. They are comparable to errors in technic in blood grouping.

Landsteiner and Levine (1928) have recently studied the inheritance of additional agglutinable elements in the blood, demonstrable only by immune serums. These they find to be Mendelian dominants. They have designated two of these as M and N, respectively, and find them to be inherited independently of the blood groups and probably independently of each

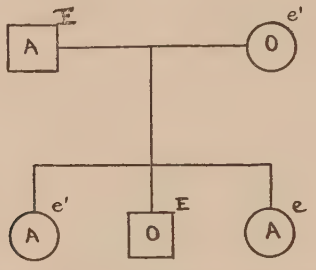

FIG. 21. FAMILY HISTORY SHOWING THE POSSIBILITY OF PREDICTING BLOOD GROUP AND EYE COLOR OF FATHER

Blood groups shown within squares and circles; E represents brown eyes, e′ represents grey eyes, e represents blue eyes.

other. Schiff (1929) has confirmed these results. Here, then, are additional factors to be used medico-legally.

Other human hereditary factors may be similarly used. By gradually accumulating such tests, the determination of disputed parentage will become more and more accurate. A man who fails in several such tests can be considered without doubt as not being the father. It must be remembered, however, that the true father can never be identified by such tests as these. It can sometimes be said that a certain man is *not* the father of the child; it can sometimes be said that he *might* be the father; but it can never be said that any man *is* the father.

Figure 21 shows a family history in which the father can be placed as to blood group and eye color from the known groups and eye colors of the mother and children. By referring to table 14 and figure 21, it can be seen that the father must be brown-eyed, and of group *A*.

In certain instances it may be possible to identify a baby, when, as occasionally happens, infants are mixed in hospitals. By knowing the blood group of each parent, it is possible to state the groups to which their child might belong. For example, if the father and mother were both group *O*, and the two infants in question were groups *O* and *A*, respectively, the baby of group *O* must be the offspring of the two parents under consideration. A list of these possibilities is given in table 15.

In making such tests, it must be remembered that very young infants may not have their final blood group fully established, as pointed out in chapter IV. After a child is a month old, however, the blood group based on the erythrocytes might safely be considered as accurate. In younger infants the test should certainly be repeated after several months.

TABLE 15

POSSIBLE BLOOD GROUPS OF CHILDREN FROM VARIOUS MATINGS

PARENTS	CHILDREN POSSIBLE	CHILDREN NOT POSSIBLE
O × O	O	A, B, AB
O × A	O, A	B, AB
A × A	O, A	B, AB
O × B	O, B	A, AB
B × B	O, B	A, AB
A × B	O, A, B, AB	—
O × AB	A, B	O, AB
A × AB	A, B, AB	O
B × AB	A, B, AB	O
AB × AB	A, B, AB	O

Another medico-legal application of blood grouping is the identification of stains. The application here consists of identifying the stain as to blood group. Of course it must first be determined by the precipitin test that the blood is actually human blood. Then the blood group of the person from whom the stain came may be determined from the stain. Lattes (1927) has presented a technic for this which is applicable to very small spots of blood, and even to old stains. He lists a series of cases in which this method was used medico-legally, some of the stains being as much as eighteen months old at the time of grouping.

Alieff (1927) presents a workable technic for this determination, based on the property of erythrocytes of absorbing agglutinins. This property is retained by the agglutinogens in the red cells of dried blood for at least eight weeks. The method is as follows: 1 gram or less of the dried blood to be

MN × MN	MN, MN	—
MN × N	MN, N	M
MN × M	MN, M	N
M × N	MN	M, N
M × M	M	MN, N
N × N	N	M, MN

examined is mixed with 1 cc. of serum of group *O*. The mixture is kept in the thermostat at 37°C. for from two to five minutes. It is then tested against corpuscles of known groups *A* and *B*. The readings are interpreted as follows. If the mixture agglutinates both the corpuscles of *A* and of *B*, the dried blood belongs to group *O*. If the mixture agglutinates the corpuscles of *B* but not those of *A*, the dried blood belongs to group *A*. If the mixture agglutinates erythrocytes of group *A* but not those of *B*, the stain belongs to group *B*. Finally, if the mixture does not agglutinate either the cells of *A* or of *B*, the dried blood belongs to group *AB*.

Here again it must be remembered that the medico-legal application is a question of eliminating certain persons, rather than incriminating any particular individual, except as may be done by such elimination.

CHAPTER XII

The Blood Groups and Pathology

"Die Blutgruppenforschung stellt im gegenwärtigen Augenblick die veilseitigste und vielleicht tiefste Anwendung der Immunitätsforschung dar, die weit über die Probleme der individuellen Pathologie hinausragt."—Hirszfeld, *Ueber die Konstitutionsserologie im zusammenhang mit der Blutgruppenforschung*, 1926.

INTRODUCTION

The demonstration of the hereditary nature of the blood groups leads directly to another problem having both theoretical and practical implications. This problem is the study of the relations of the blood groups to other human characters. The blood groups may have a definite influence on pathologic, physiologic, or morphologic conditions, due to linkage relations or other effects. Hereditary characters carried on the same pair of chromosomes as the blood groups would be linked with the blood groups in inheritance. This possibility opens a wide range of speculation from a pathologic standpoint. Hereditary characters carried on other chromosomes would be inherited independently of the blood groups. Non-hereditary factors could be only indirectly influenced by the hereditary nature of the groups.

This general subject has been investigated by many workers of late. Several investigators have concluded that the particular character under discussion was linked with the groups in inheritance. All of these conclusions are based on false premises or erroneous ideas of linkage. In order to simplify further inquiry into the linkage relations of the blood groups, and to place such inquiries on common ground, I am including tables showing the possible linkage relationships of the blood groups and any other pair of hereditary factors (see tables 16 and 17).

Two things must be borne in mind in any study of the linkage relations of the blood groups. First, linkage can never be demonstrated by means of abnormal distribution of the four groups among persons showing a particular character. Second, owing to the difficulties inherent in the study of human heredity, all family histories involving the simultaneous inheritance of two pairs of characters will not give data on linkage. In animals, in which controlled matings make possible the determination of the genetic formula of any individual, and in which large numbers of offspring may be

raised from any desired type of cross, studies of linkage are comparatively easy. In man, however, it is difficult to determine the genetic constitution, and controlled matings are not available; the problem therefore becomes more difficult. In many families it is almost impossible to determine whether the coupling phase or the repulsion phase is involved, and therefore which of the offspring are cross-overs and which are non-cross-overs (see fig. 18). It is likewise practically impossible to be sure in certain instances that a dominant parent is homozygous, or, as is more to the point, to know whether a dominant parent is heterozygous in the absence of recessive offspring.

THEORETICAL CONSIDERATIONS

In considering the relations of the blood groups to other characters there are five possibilities:

1. The possibility of complete linkage of some hereditary factor with one of the blood group factors. In this case, if the mutation causing the character had taken place but once, the character, if recessive, would be confined to group *O*, or to group *A*, or to group *B*, and would not appear in any of the others. If dominant, the character might also appear in group *AB*. For example, if a recessive mutation causing feeble-mindedness had taken place on a chromosome carrying the blood group factor A, all feebleminded persons would be of group *A*. As a matter of fact, however, feebleminded persons occur in all four groups (see table 18). Even so, there might still be complete linkage if the mutation had taken place several times, at least once on a chromosome carrying the blood group factor A, once on a chromosome carrying the factor B, and once on a chromosome carrying the factor O. In such a case, inheritance studies would quickly reveal the fact of complete linkage (see tables 16 and 17). Complete linkage is hardly to be expected, however, and the next possibility is of much more importance.

2. The possibility of partial linkage of some hereditary factor with the blood group factors. In this case, although the original mutation would have been linked with but one of the blood group factors, subsequent crossing over would in time distribute the factor proportionately among the four groups. Therefore linkage can never be detected by observing the percentages of the four groups among various morphologic or pathologic conditions. This fundamental fact has not been appreciated by many investigators. Thus, claims of linkage based on abnormal distributions can have no logical basis.

When partial linkage occurs, it may be demonstrated only by careful pedigrees for at least two generations. When these are traced, the data will in some cases lend themselves to analysis from the standpoint of linkage. All family histories will not furnish data which can be used, however.

In many matings, there can be no demonstrable difference between the results in complete linkage and those in partial linkage or independent assortment. When only two generations are studied, unless all dominant parents show some recessive children, no data on linkage is of value, since a single human family is never large enought to remove all possibility of the parents being heterozygous when they give all dominant children. For example, considering susceptibility to diphtheria as recessive to non-susceptibility, all families must show at least one susceptible child before being of value as linkage data. In the case of the blood groups themselves, all matings must show at least one child of group *O*, except where one parent is group *AB*, in which case at least one child of each of groups *A* and *B* must be present.

When three generations are studied, it may be possible in some cases to show that a dominant parent is heterozygous because one grandparent was dominant, the other recessive. In any case, sufficient combinations must appear in the offspring to give some basis for deciding between linkage and independent assortment. The minimum number of combinations will vary with the mating, and may be determined for the various types from tables 16 and 17.

3. The possibility of independent inheritance of the blood group factors and a particular hereditary factor. This possibility is most often to be expected, because of the large number of chromosomes in man, and will give all possible combinations in inheritance, in typical dihybrid ratios, as shown in the right hand columns of tables 16 and 17.

4. The possibility that one or another of the blood group factors may in addition tend to affect indirectly some morphologic, physiologic or pathologic condition, thus causing an abnormal distribution of the groups in the particular condition affected. Although not probable, this is a possibility which must be considered. In such a case, the condition affected, if hereditary, would be limited by the blood groups, and would give abnormal hereditary behavior. If not hereditary *per se*, the condition would become imperfectly hereditary by reason of its partial dependence on the blood groups. As a hypothetical example, one may assume that the blood group factor A also effects the red cells in such a way that they more readily resist the entrance of the malarial parasite. Then groups *A* and *AB* would not be so well represented among malarial patients, while groups *O* and *B* would be represented in greater proportion than they are found among the general population. The finding of this abnormal distribution of the groups in a pathologic condition would not be a basis for concluding that the condition was an inherited character linked with the groups, however. On the contrary,

TABLE 16

THEORETICAL RELATIONS OF THE BLOOD GROUP FACTORS AND ANY OTHER PAIR OF FACTORS (S AND s)

One parent dominant (−), the other recessive (+). All dominants considered heterozygous

COMPLETE LINKAGE				INDEPENDENT ASSORTMENT			
O− (OS) (Os)	×		*O*+ (Os) (Os)	*O*− OOSs	×		*O*+ OOss
	OOSs	*O*−	no difference		OOSs	*O*−	
	OOss	*O*+			OOss	*O*+	
O− (OS) (Os)	×		*A*+ (As) (Os)	*O*− OOSs	×		*A*+ AOss
	AOSs	*A*−			AOSs	*A*−	
	AOss	*A*+	no difference		AOss	*A*+	
	OOSs	*O*−			OOSs	*O*−	
	OOss	*O*+			OOss	*O*+	
O+ (Os) (Os)	×		*A*− (AS) (Os)	*O*+ OOss	×		*A*− AOSs
	AOSs	*A*−			AOSs	*A*−	
	OOss	*O*+			AOss	*A*+	
					OOSs	*O*−	
(Os) (Os)	×		(As) (OS)		OOss	*O*+	
	AOss	*A*+					
	OOSs	*O*−					
A− (AS) (Os)	×		*A*+ (As) (Os)	*A*− AOSs	×		*A*+ AOss
	AASs, AOSs	2*A*−			AASs, 2AOSs	3*A*−	
	AOss	1*A*+			AAss, 2AOss	3*A*+	
	OOss	1*O*+			OOSs	1*O*−	
(As) (OS)	×		(As) (Os)		OOss	1*O*+	
	AAss, AOss	2*A*+					
	AOSs	1*A*−					
	OOSs	1*O*−					
O− (OS) (Os)	×		*B*+ (Bs) (Os)	*O*− OOSs	×		*B*+ BOss
	BOSs	*B*−			BOSs	*B*−	
	BOss	*B*+	no difference		BOss	*B*+	
	OOSs	*O*−			OOSs	*O*−	
	OOss	*O*+			OOss	*O*+	

TABLE 16—*Continued*

COMPLETE LINKAGE				INDEPENDENT ASSORTMENT			
O+			*B*−	*O*+			*B*−
(Os) (Os)	×		(BS) (Os)	OOss	×		BOSs
	BOSs	*B*−			BOSs	*B*−	
	OOss	*O*+			BOss	*B*+	
					OOSs	*O*−	
(Os) (Os)			(Bs) (OS)		OOss	*O*+	
	BOss	*B*+					
	OOSs	*O*−					
A−			*B*+	*A*−			*B*+
(AS) (Os)	×		(Bs) (Os)	AOSs	×		BOss
	ABSs	*AB*−			ABSs	*AB*−	
	AOSs	*A*−			ABss	*AB*+	
	BOss	*B*+			BOSs	*B*−	
	OOss	*O*+			BOss	*B*+	
					AOSs	*A*−	
(As) (OS)	×		(Bs) (Os)		AOss	*A*+	
	ABss	*AB*+			OOSs	*O*−	
	AOss	*A*+			OOss	*O*+	
	BOSs	*B*−					
	OOSs	*O*−					
A+			*B*−	*A*+			*B*−
(As) (Os)	×		(BS) (Os)	AOss	×		BOSs
	ABSs	*AB*−			ABSs	*AB*−	
	AOss	*A*+			ABss	*AB*+	
	BOSs	*B*−			BOSs	*B*−	
	OOss	*O*+			BOss	*B*+	
					AOSs	*A*−	
(As) (Os)	×		(Bs) (OS)		AOss	*A*+	
	ABss	*AB*+			OOSs	*O*−	
	AOSs	*A*−			OOss	*O*+	
	BOss	*B*+					
	OOSs	*O*−					
B−			*B*+	*B*−			*B*+
(BS) (Os)	×		(Bs) (Os)	BOSs	×		BOss
	BBSs, BOSs	2*B*−			BBSs, 2BOSs	3*B*−	
	BOss	1*B*+			BBss, 2BOss	3*B*+	
	OOss	1*O*+			OOSs	1*O*−	
					OOss	1*O*+	
(Bs) (OS)	×		(Bs) (Os)				
	BBss, BOss	2*B*+					
	BOSs	1*B*−					
	OOSs	1*O*−					

TABLE 16—*Continued*

COMPLETE LINKAGE				INDEPENDENT ASSORTMENT			
O− (OS) (Os)	×		*AB*+ (As) (Bs)	*O*− OOSs	×		*AB*+ ABss
AOSs	*A*−			AOSs	*A*−		
AOss	*A*+	no difference		AOss	*A*+		
BOSs	*B*−			BOSs	*B*−		
BOss	*B*+			BOss	*B*+		
O+ (Os) (Os)	×		*AB*− (AS) (Bs)	*O*+ OOss	×		*AB*− ABSs
AOSs	*A*−			AOSs	*A*−		
BOss	*B*+			AOss	*A*+		
				BOSs	*B*−		
(Os) (Os)	×		(As) (BS)	BOss	*B*+		
AOss	*A*+						
BOSs	*B*−						
A− (AS) (Os)	×		*AB*+ (As) (Bs)	*A*− AOSs	×		*AB*+ ABss
AASs	*A*−			AASs, AOSs		2*A*−	
AOss	*A*+			AAss, AOss		2*A*+	
ABSs	*AB*−			ABSs		1*AB*−	
BOss	*B*+			ABss		1*AB*+	
(As) (OS)	×		(As) (Bs)	BOSs		1*B*−	
AAss	*A*+			BOss		1*B*+	
AOSs	*A*−						
ABss	*AB*+						
BOSs	*B*−						
A+ (As) (Os)	×		*AB*− (AS) (Bs)	*A*+ AOss	×		*AB*− ABSs
AASs, AOSs		2*A*−		AASs, AOSs		2*A*−	
ABss		1*AB*+		AAss, AOss		2*A*+	
BOss		1*B*+		ABSs		1*AB*−	
(As) (Os)	×		(As) (BS)	ABss		1*AB*+	
AAss, AOss		2*A*+		BOSs		1*B*−	
ABSs		1*AB*−		BOss		1*B*+	
BOSs		1*B*−					

TABLE 16—*Concluded*

COMPLETE LINKAGE			INDEPENDENT ASSORTMENT		
B− (BS) (Os)	×	*AB*+ (As) (Bs)	*B*− BOSs	×	*AB*+ ABss
ABSs	*AB*−		ABSs	1*AB*−	
AOss	*A*+		ABss	1*AB*+	
BBSs	*B*−		AOSs	1*A*−	
BOss	*B*+		AOss	1*A*+	
			BBSs, BOSs	2*B*−	
(Bs) (OS)	×	(As) (Bs)	BBss, BOss	2*B*+	
ABss	*AB*+				
AOSs	*A*−				
BBss	*B*+				
BOSs	*B*−				
B+ (Bs) (Os)	×	*AB*− (AS) (Bs)	*B*+ BOss	×	*AB*− ABSs
ABSs	1*AB*−		ABSs	1*AB*−	
AOSs	1*A*−		ABss	1*AB*+	
BBss, BOss	2*B*+		AOSs	1*A*−	
			AOss	1*A*+	
			BBSs, BOSs	2*B*−	
(Bs) (Os)	×	(As) (BS)	BBss, BOss	2*B*+	
ABss	1*AB*+				
AOss	1*A*+				
BBSs, BOSs	2*B*−				
AB− (AS) (Bs)	×	*AB*+ (As) (Bs)	*AB*− ABSs	×	*AB*+ ABss
AASs	*A*−		AASs	1*A*−	
ABSs	*AB*−		AAss	1*A*+	
ABss	*AB*+		2ABSs	2*AB*−	
BBss	*B*+		2ABss	2*AB*+	
			BBSs	1*B*−	
(As) (BS)	×	(As) (Bs)	BBss	1*B*+	
AAss	*A*+				
ABss	*AB*+				
ABSs	*AB*−				
BBSs	*B*−				

TABLE 17

THEORETICAL RELATIONS OF THE BLOOD GROUP FACTORS AND ANY OTHER PAIR OF FACTORS (S AND s)

Both parents dominant (−). All dominants considered heterozygous

COMPLETE LINKAGE			INDEPENDENT ASSORTMENT		
O− (OS) (Os)	×	*O*− (OS) (Os)	*O*− OOSs	×	*O*− OOSs
OOSS, 2OOS		3*O*−	OOSS, 2OOSs		3*O*−
OOss		1*O*+	OOss		1*O*+
O− (OS) (Os)	×	*A*− (AS) (Os)	*O*− OOSs	×	*A*− AOSs
AOSS, AOSs		2*A*−	AOSS, 2AOSs		3*A*−
OOSs		1*O*−	AOss		1*A*+
OOss		1*O*+	OOSS, 2OOSs		3*O*−
			OOss		1*O*+
(OS) (Os)	×	(As) (OS)			
AOSs		1*A*−			
AOss		1*A*+			
OOSS, OOSs		2*O*−			
A− (AS) (Os)	×	*A*− (AS) (Os)	*A*− AOSs	×	*A*− AOSs
AASS, 2AOSs		3*A*−	AASS, 2AOSS, 2AASs, 4AOSs		9*A*−
OOss		1*O*+	AAss, 2AOss		3*A*+
(AS) (Os)	×	(As) (OS)	OOSS, 2OOSs		3*O*−
AOss		1*A*+	OOss		1*O*+
AASs, AOSS		2*A*−			
OOSs		1*O*−			
(As) (OS)	×	(As) (OS)			
AAss		1*A*+			
2AOSs		2*A*−			
OOSS		1*O*−			

TABLE 17—*Continued*

COMPLETE LINKAGE			INDEPENDENT ASSORTMENT		
O−		*B*−	*O*−		*B*−
(OS) (Os)	×	(BS) (Os)	OOSs	×	BOSs
BOSS, BOSs		2*B*−	BOSS, 2BOSs		3*B*−
OOSs		1*O*−	BOss		1*B*+
OOss		1*O*+	OOSS, 2OOSs		3*O*−
			OOss		1*O*+
(OS) (Os)	×	(Bs) (OS)			
BOSs		1*B*−			
BOss		1*B*+			
OOSS, OOSs		2*O*−			
A−		*B*−	*A*−		*B*−
(AS) (Os)	×	(BS) (Os)	AOSs	×	BOSs
ABSS		*AB*−	ABSS, 2ABSs		3*AB*−
AOSs		*A*−	ABss		1*AB*+
BOSs		*B*−	AOSS, 2AOSs		3*A*−
OOss		*O*+	AOss		1*A*+
(AS) (Os)	×	(Bs) (OS)	BOSS, 2BOSs		3*B*−
ABSs		*AB*−	BOss		1*B*+
AOSS		*A*−	OOSS, 2OOSs		3*O*−
BOss		*B*+	OOss		1*O*+
OOSs		*O*−			
(As) (OS)	×	(BS) (Os)			
ABSs		*AB*−			
AOss		*A*+			
BOSS		*B*−			
OOSs		*O*−			
(As) (OS)	×	(Bs) (OS)			
ABss		*AB*+			
AOSs		*A*−			
BOSs		*B*−			
OOSS		*O*−			

TABLE 17—*Continued*

COMPLETE LINKAGE			INDEPENDENT ASSORTMENT		
B−		*B*−	*B*−		*B*−
(BS) (Os)	×	(BS) (Os)	BOSs	×	BOSs
BBSS, 2BOSs		3*B*−	BBSS, 2BBSs, 2BOSS, 4BOSs		9*B*−
OOss		1*O*+	BBss, 2BOss		3*B*+
(Bs) (OS)	×	(Bs) (OS)	OOSS, 2OOSs		3*O*−
BBss		1*B*+	OOss		1*O*+
2BOSs		2*B*−			
OOSS		1*O*−			
(BS) (Os)	×	(Bs) (OS)			
BBSs, BOSS		2*B*−			
BOss		1*B*+			
OOSs		1*O*−			
O−		*AB*−	*O*−		*AB*−
(OS) (Os)	×	(AS) (Bs)	OOSs	×	ABSs
AOSS, AOSs		2*A*−	AOSS, 2AOSs		3*A*−
BOSs		1*B*−	AOss		1*A*+
BOss		1*B*+	BOSS, 2BOSs		3*B*−
(OS) (Os)	×	(As) (BS)	BOss		1*B*+
AOSs		1*A*−			
AOss		1*A*+			
BOSS, BOSs		2*B*−			
A−		*AB*−	*A*−		*AB*−
(AS) (Os)	×	(AS) (Bs)	AOSs	×	ABSs
AASS, AOSs		2*A*−	AASS, 2AASs, AOSS, 2AOSs		6*A*−
ABSs		1*AB*−	AAss, AOss		2*A*+
BOss		1*B*+	ABSS, 2ABSs		3*AB*−
(As) (OS)	×	(AS) (Bs)	ABss		1*AB*+
AASs, AOSS		2*A*−	BOSS, 2BOSs		3*B*−
ABss		1*AB*+	BOss		1*B*+
BOSs		1*B*−			
(AS) (Os)	×	(As) (BS)			
AASs		*A*−			
AOss		*A*+			
ABSS		*AB*−			
BOSs		*B*−			
(As) (OS)	×	(As) (BS)			
AAss		*A*+			
AOSs		*A*−			
ABSs		*AB*−			
BOSs		*B*−			

TABLE 17—*Concluded*

COMPLETE LINKAGE			INDEPENDENT ASSORTMENT		
B− (BS) (Os)	×	*AB*− (AS) (Bs)	*B*− BOSs	×	*AB*− ABSs
ABSS		*AB*−	BBSS, 2BBSs, BOSS, 2BOSs		6*B*−
BBSs		*B*−			
BOss		*B*+	BBss, BOss		2*B*+
AOSs		*A*−			
(Bs) (OS)	×	(AS) (Bs)	ABSS, 2ABSs		3*AB*−
ABSs		*AB*−	ABss		1*AB*+
BBss		*B*+	AOSS, 2AOSs		3*A*−
AOSS		*A*−	AOss		1*A*+
BOSs		*B*−			
(BS) (Os)	×	(As) (BS)			
ABSs		1*AB*−			
BBSS, BOSs		2*B*−			
AOss		1*A*+			
(Bs) (OS)	×	(As) (BS)			
ABss		1*AB*+			
BBSs, BOSS		2*B*−			
AOSs		1*A*−			
AB− (AS) (Bs)	×	*AB*− (AS) (Bs)	*AB*− ABSs	×	*AB*− ABSs
AASS		1*A*−	2ABSS, 4AVSs		6*AB*−
2ABSs		2*AB*−	2ABss		2*AB*+
BBss		1*B*+	AASS, 2AASs		3*A*−
(AS) (Bs)	×	(As) (BS)	AAss		1*A*+
AASs		*A*−	BBSS, 2BBSs		3*B*−
ABSs		*AB*−	BBss		1*B*+
ABss		*AB*+			
BBSs		*B*−			
(As) (BS)	×	(As) (BS)			
AAss		1*A*+			
2ABSs		2*AB*−			
BBSS		1*B*−			

as has already been pointed out, if there were true linkage, the distribution of the groups would be normal.

5. A fifth possibility, much restricted and hypothetical, may be mentioned. If we accept the serologic basis necessary on the triple allelomorph hypothesis, namely, that all serums are essentially similar as to iso-agglutinins, we might well expect disarrangements of some sort in children at the onset of the agglutinin. In this case the effect of the reciprocal binding would be most marked in children of group *AB*, and would not occur at all in children of group *O*. This phenomenon, if it occurs, must be restricted to sudden, apparently spontaneous disarrangements in children of groups *A*, *B*, and *AB*, from birth to about two years. It may be stated in passing that my preliminary study of convulsions in children has not borne out this possibility.

In deciding which of the above possibilities is involved in any study of the relations of the blood groups to other characters, tables 16 and 17 will be found of value. The left hand columns of these tables give the results to be expected in complete linkage. In all probability this would rarely occur. The right hand columns of the tables give the results to be expected in independent assortment. Between these two extremes would be the results to be expected in cases of partial linkage, varying towards one extreme or the other with the strength of the linkage. In these tables the three allelomorphic blood group factors have been represented by the customary genetic designations A, B, and O, and the groups themselves by italicized *O*, *A*, *B*, and *AB*. The pair of factors whose linkage relations are being studied are empirically represented by S and s, and their phenotypes by − (dominant) and + (recessive), respectively. All dominants are considered heterozygous, as otherwise no linkage could be studied.

From these tables certain rules may be formed. For example, in table 16, in the mating of group *O*+ with group *A*−, if *A*+ and *A*−, or *O*+ and *O*− occur in the offspring of the same family, crossing over has taken place. If this occurs in practically every family of this kind, independent assortment may be assumed; if in only a very few families of this nature, linkage may be assumed. The exact strength of the linkage would be very hard to determine, and must be based on mass statistics.

As another example, in table 17, in the mating of *A*− with *B*−, the + offspring must all belong to the same group. If they belong to more than one group, within the same family, crossing over has taken place.

Rules such as these may be formed for many of the possible matings. In some cases, such as those where both parents are group *O*, or in matings of *O*− with *A*+, and similar combinations, there would be no discernable difference between the results of linkage and free assortment.

BLOOD GROUP DISTRIBUTION

In studying the relationships of blood groups to other characters, then, two procedures are available. The first is to study the distribution of the groups among persons showing some specific condition, such as cancer or malaria. Results from such studies will indicate whether or not the fourth possibility mentioned above occurs, that is, whether the condition studied is indirectly affected by the blood groups. Of course the distribution must be compared with the normal distribution in the particular people being studied. Such studies may in general be expected to yield negative results, that is the distribution may be expected to be normal in most cases. It is important to continue these investigations, however, as long as material is available.

The second procedure, equally valuable but for a different reason, is to study the simultaneous inheritance of the blood groups and other heritable characters in two or more generations. By accumulating large numbers of accurate family histories, valuable results may be expected. These two lines of procedure have been carried out by various investigators, and will be discussed in some detail.

Before any abnormal distribution of blood groups can be accepted as certain, large numbers of cases must be studied. The small numbers which have been reported by so many investigators may easily be chance variations. Thus Alexander (1921) in a study of fifty cases of malignant disease, found that group *O* was not so well represented as among the general population, while groups *B* and *AB* were better represented. Buchanan and Higley (1921) and Pfahler and Widmann (1922), however, found all groups represented in proportions similar to those occurring in healthy people of the same nationality. Johannsen (1925) stated that group *O* was represented less frequently than in normal persons, but the discrepancy which he cites is slight. Weitzner (1925) found among 84 cases of carcinoma considerably less group *O* blood than normal, and considerably more group *AB* blood. Schiff (1926), however, takes exception to these results, stating that patients with cancer show the same proportions of the blood groups as healthy people from the same locality. He considers that Weitzner's results are due to error from pseudo-agglutination, which is of frequent occurrence in the blood of patients suffering from cancer. Hoche and Moritsch (1926) found an abnormally large percentage of group *AB* among 176 cases of cancer. Bendien (1926) found in 110 patients with cancer a low percentage of group *O* and a high percentage of group *AB*. Hirszfeld and Hittmair (1926) on the other hand found a normal distribution among 150 cases of cancer.

My own results to date show a normal distribution. Until the possibility of pseudo-agglutination is ruled out, the abnormal distributions can not be accepted as proven. It will be noted that in those cases in which an increase of some group is reported, the increase is always in the direction of more agglutination (group *AB*), away from the lack of agglutination (group *O*). This fact, taken in consideration with the known frequency of pseudo-agglutination in the bloods of cancer patients, makes controls such as kaolin or lecithin absolutely necessary. It is advisable to check all blood groups of cancer patients with tests of their serums.

Studying syphilis in its relations with the blood groups, both Straszynski (1925) and Wiechmann and Paal (1926) found group *O* poorly represented among cases in which the Wassermann reaction was positive, while group *AB* was overly represented. In addition, Straszynski found that a larger percentage of group *O* patients recovered under treatment. He concluded that the rapidity of disappearance of the Wassermann reaction is a constitutional character linked in inheritance with the blood groups, a conclusion which is untenable when examined in the light of the discussion on linkage given earlier in this chapter. Gundel (1927, 1928) found no abnormal distribution among 2665 persons with positive Wassermann reaction. He was able to confirm Straszynski's observations that groups *B* and *AB* are harder to influence by treatment, however. Diamantopoulos (1928) found no abnormal distribution among 405 cases of syphilis. Naito (1928) found no relationship of any kind between the blood groups and the Wassermann reaction. My own results to date show no significant abnormal distribution.

Some work has been done on the blood groups in their relation to the various complications in pregnancy. Schneider (1925) stated that toxemia did not accompany pregnancy when the group of the mother and that of the child coincided. He considers that a divergence in blood groups causes a disposition towards eclampsia. Hirszfeld and Zborowski (1926) believe that considerable differences in the blood groups of husband and wife may retard the developing embryo, and that extreme differences may even affect fecundity. They go so far as to suggest certain groups which may be sterile when mated together. At least part of their conclusions are rendered invalid when their material is considered from the standpoint of the triple allelomorph hypothesis of heredity, and several investigators (Rech and Wöhlisch 1926, Collon 1927, Thomsen 1928) take direct exception to these results.

Cathala and LeRasle (1925) concluded that incompatibility of maternal and fetal blood is not responsible for eclampsia or other complications in

pregnancy. Ohnesorge (1925) found that incompatibility of groups of mother and child has no connection with toxicoses of pregnancy. Allen (1926) found that the percentages of incompatibility between the blood of the mother and that of the infant were similar in normal women and in cases of late toxemias of pregnancy. He concluded that there was no evidence that the late toxemias of pregnancy have their origin in the iso-agglutination phenomena.

In a study of the blood groups of infants, Smith (1928) concluded that incompatibility of blood group between mother and child probably does not play a primary rôle in the etiology of icterus neonatorum. Interagglutination may serve, however, as an accessory factor in the production of this condition.

Kaczynsky (1926) found that the percentage of persons susceptible to the Dick test is approximately the same in each of the four groups. The same is true of the Schick test. Holló and Lénard (1926), Alperin (1926), Kochs and Wilcken (1928), and others have found a normal distribution of the groups among tuberculosis patients. This is confirmed by my own observations. Hermanns and Kronberg (1927) found among other things a relation between blood groups and hyperthyroidism. Kraus and Medvei (1929) tested this claim, but could find no abnormal distribution at all. Hilgers, Wohlfeil, and Knötzke (1928) found a slightly higher porportion of group *O* among cases of positive Gruber-Widal reaction.

Mironescu and Stefanoy (1926) gave figures on the percentages of the blood groups among persons with various diseases, but the numbers are entirely too small to be of much value. Chi-Pan (1924), Leisermann (1927), Diamantopoulos (1928), Perkel and Israelsson (1928), Solojew and Winigradowa (1928) and others, have recorded normal distributions in various diseases (typhoid, typhus, tuberculosis, malaria, syphilis, whooping cough, measles, scarlet fever, and other pathologic conditions).

Feldmann and Elmanowitsch (1925) found a high percentage of group *AB* in 815 cases of mental disease. In paretic patients the occurence of *AB* rose as high as 33 per cent. These authors consider the blood groups as valuable aids in psychiatric diagnosis. Gundel (1926) reported an increased percentage of group *B* in a series of 402 cases of mental diseases. My own results in these conditions, however (Snyder 1926), showed normal distributions in several hundred cases of epilepsy, dementia praecox, and feeble-mindedness. Subsequent amplification of these results in my laboratory have confirmed the original findings.

Jacobsohn (1928) found the blood group distribution normal among patients with general paresis. Gundel (1926) reported a normal distribution

among criminals. Böhmer (1927), however, found an increased percentage of *B* (20 per cent) among 1034 criminals. Wilczowski (1927) found an excess of group *AB* among patients with paresis, but a normal distribution among patients with schizophrenia. Bunker and Meyers (1927) and Pilcz (1927) reported that the groups were normally distributed among a series of patients with general paresis. On the other hand, Proescher and Arkush (1927) reported that in a series of 2104 cases from the California State Hospital, groups *O* and *B* were more susceptible to insanity (twice as susceptible as group *A* and four times as susceptible as group *AB*). This relation held good not only for insanity in general, but for the individual conditions of dementia praecox, maniac depression, general paresis, epilepsy, and others. Meyer (1928) found an increased percentage of *B* in maniac depressives, and of *A* in progressive paretics.

TABLE 18

DISTRIBUTION OF THE BLOOD GROUPS AMONG NORMAL AMERICANS AND IN SOME PATHOLOGIC CONDITIONS

CONDITION	NUMBER STUDIED	PER CENT IN GROUP			
		O	*A*	*B*	*AB*
Normal	20,000	45	41	10	4
Dementia praecox	200	45	42	9	4
Epilepsy	150	48	40	10	2
Feeble-mindedness	300	44	42	10	4
Malaria	100	47	30	18	5
Syphilis	100	44	42	8	6
Migraine	500	47	40	7	6
Polydactylism (negroes)	50	49	28	19	4

Thus it is seen that conflicting reports constantly occur, some workers claiming an excess of group *A*, some of group *B*, some of group *AB*, and still others of group *O*, while many investigators find a normal distribution. Under such conditions, no abnormal distribution can as yet be accepted as final, and it seems likely that a normal distribution will eventually be demonstrated for these conditions.

Percentages of the blood groups among persons of the same race differing in pigmentation, in color of hair, in form of head, and in body build have been recorded, but without showing any significant differences (Hirszfeld and Hirszfeld 1919, Dossena 1924, 1925, Klein and Osthoff 1926, Steffan 1926–29, et al.). A few figures from my own laboratory showing the normal distribution of the groups in various conditions are given in table 18.

FAMILY HISTORIES

Very few investigators have studied linkage by the only correct technic, that is, by family histories involving the simultaneous inheritance of the blood groups and other human characters. Hirszfeld, Hirszfeld and Brokman (1924) presented family histories of the inheritance of the blood groups and susceptibility to the Schick test. They concluded that susceptibility to diphtheria was linked with the blood groups. On this basis Hoche and Moritsch (1925) have assumed that from a knowledge of

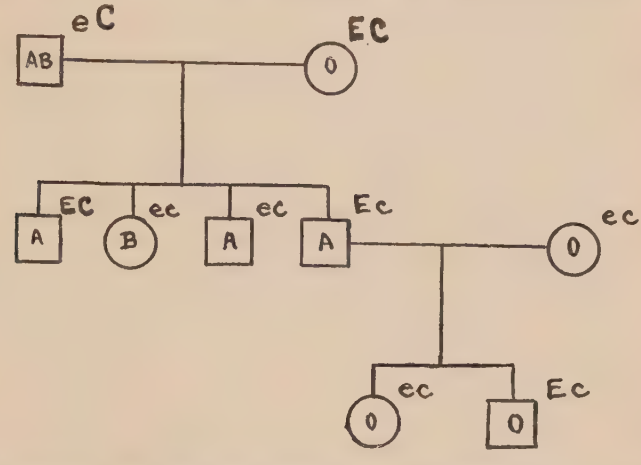

FIG. 22. FAMILY HISTORY INVOLVING THE SIMULTANEOUS INHERITANCE OF BLOOD GROUPS, EYE COLOR, AND DIRECTION OF OCCIPITAL HAIR WHORL, SHOWING CROSSING-OVER BETWEEN EACH TWO PAIRS OF FACTORS

Blood groups shown within the squares and circles; E represents brown eyes, e represents blue eyes; C represents clockwise hair whorl, c represents counter-clockwise hair whorl.

the blood groups of a family and the Schick reactions of the parents, accurate knowledge of the susceptibility of the children can be obtained. Such assumptions are unwarranted, and may be dangerous to life. In the first place, even if there were linkage, crossing-over would obviate the possibility of making practical clinical applications, as will be pointed out later. In the second place, the family histories in question give no more indication of linkage than they do of free assortment. Of the 42 families in question, 11 must be discarded as linkage data because both parents are homozygous for a recessive factor. Thirteen of the families must be discarded because

the lack of recessive children withholds any evidence that the dominant parents are heterozygous. Six more must be discarded because they could give no difference between linkage and free assortment even if all other conditions were fulfilled. Of the remaining 11 families, 3 definitely show crossing-over. The other 8 could indicate either linkage or crossing-over, but the number of offspring is too small to be of much significance, 2 of the families having but one child each. There is thus no basis for concluding linkage of the blood groups with diphtheria susceptibility.

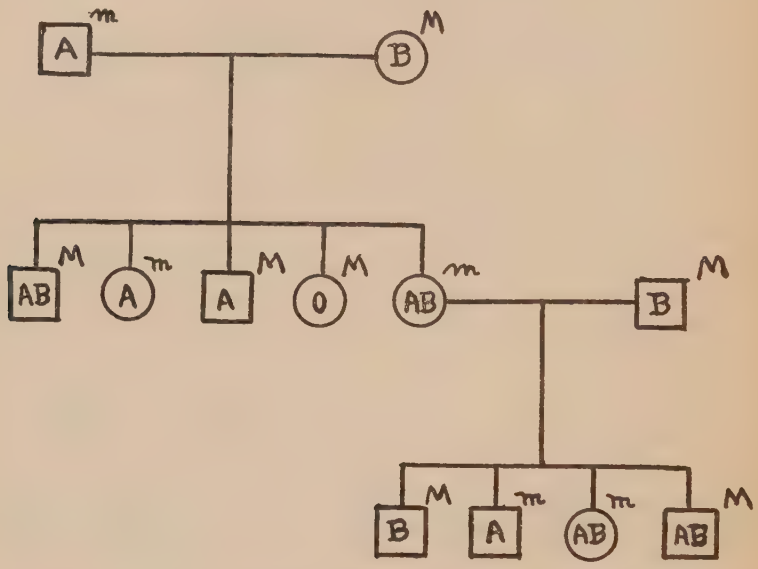

FIG. 23. FAMILY HISTORY ILLUSTRATING CROSSING-OVER BETWEEN THE BLOOD GROUPS AND MIGRAINE

Blood groups shown within the squares and circles; M represents migraine, m represents normal.

It is conceivable that although no linkage is concerned, the blood group factors might indirectly affect susceptibility to diphtheria in a manner such as that outlined above under possibility 4. In this event, however, an abnormal distribution of the groups among susceptible patients would be expected, a condition which does not occur.

This case has been considered in some detail because it is indicative of the faulty basis for assuming linkage used by various writers. Thus Fürst (1925) on equally untenable grounds assumed that susceptibility to goiter

was linked with the blood groups. Kubányi (1926) concluded that there was linkage between the blood groups and hemophilia. Since hemophilia is a sex-linked character carried by the sex chromosomes, this is obviously impossible.

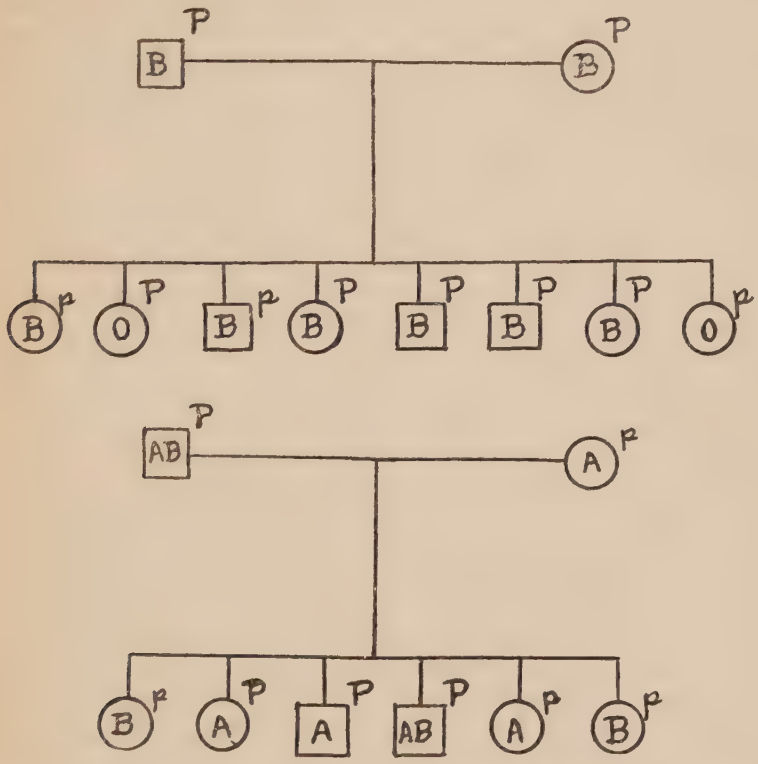

FIG. 24. FAMILY HISTORY ILLUSTRATING CROSSING-OVER BETWEEN THE BLOOD GROUPS AND POLYDACTYLISM

Blood groups shown within the squares and circles; P represents the normal condition, p represents polydactylism.

Levine (1926) in a very careful study of the relationships of the blood groups and atopic hypersensitiveness, found that there was no evidence for linkage of the character with the blood groups.

Earlier reports of my own (1926, 1927, 1929) have shown the independent inheritance of the blood groups and various factors. Many hundreds of family histories are necessary in order to have data enough for a comprehen-

sive study. So far I have been able to affirm the independent inheritance of the blood groups and migraine, polydactylism, eye color, direction of occipital hair whorl, and probably feeble-mindedness. Pedigrees illustrating crossing-over between the blood groups and some of these conditions are given in figures 22, 23, and 24. This work was done under a grant from the American Medical Association.

My results indicate that migraine is a Mendelian dominant, confirming the results of Allan (1928) with whom I have worked in collecting migraine pedigrees such as those given in figure 24.

APPLICATIONS TO CLINICAL MEDICINE

What is the significance of such actual or potential results in clinical medicine? Is it of any advantage from a clinical standpoint to know whether or not a particular pathological condition is linked with the blood groups? If we examine tables 16 and 17 we observe that there is no difference, when a large number of families are considered together statistically, between complete linkage and independent assortment, the extremes of possibility. There is a difference, however, between linkage and independent assortment when the individual families are considered separately. Suppose that we could show that migraine was always linked with group *A*, that is, that the factor causing migraine was on the chromosome containing factor A, and so close to factor A as to preclude crossing-over. It would be possible in such a case to predict from a knowledge of the blood groups and migraine history of the parents, which of the children would have migraine in later life. As migraine does not occur until adolescence or later, this would be very valuable information. However, the possibility of such an occurrence is infinitely remote. It could only be so if the mutation causing migraine had taken place on no chromosome of the blood group pair except one containing the factor A, an unwarranted assumption. Even if such a condition had arisen, a further necessity would be that the locus of the migraine mutation be so close to that of factor A as to preclude all crossing over. The slightest amount of crossing over acting over a period of years would distribute the mutation evenly among the blood group factors, making it impossible to tell whether the coupling phase or the repulsion phase was involved in any family. In other words, it would be impossible to know which possibility on the left hand columns of tables 16 and 17 was involved in a particular family of any type of mating.

It may be seen then, that while the linkage relations of the blood groups are of great interest from a genetic standpoint, there would be little practical value from a clinical standpoint in knowing the linkage relations of the blood

groups and other hereditary factors, pathological or physiological. From a practical clinical point of view, possibility 4 as outlined above is the only one apt to be of value. It is important then, to continue investigating the distribution of the blood groups among various conditions. It is not proper, however, to consider these as linkage studies, nor to conclude that linkage is being determined, as so many writers have done. It is also important from a genetic standpoint to continue the true linkage studies by the family history method, theoretical though the importance may seem at the present time. Only thus is knowledge of any subject rounded out. Only thus are broad foundations laid. And who may venture to predict the practical applications which find their bases in facts which at first seemed only of theoretical importance?

CHAPTER XIII

Blood Groups in Animals

"The problem of man's kinship to his closest relatives in the animal kingdom has been studied by the methods of comparative anatomy and paleontology and the results of these investigations are developing into a special branch of learning. When serology provided a new technic for recognizing the biochemical properties which characterize species it became inevitable that the technic should be applied to the problem of man's ancestry."—Landsteiner and Miller, *Serological Studies on the Blood of the Primates*, 1925.

The question of the occurrence of blood groups in the lower animals has never been satisfactorily settled. Most investigators have failed to demonstrate fixed groupings in animals below the anthopoid apes. Occasional workers, however, have reported the finding of iso-agglutinins and iso-agglutinogens, on the basis of which groups have been formed.

The possible presence of blood groups in animals is of interest from several standpoints. The question of the mode of inheritance of the groups might be attacked experimentally, were groups similar to the human blood groups to be discovered in a laboratory animal. The taxonomic relationships of animals might be better understood if the morphologic findings could be supplemented by this serologic approach. And finally, the problem of the origin and evolutionary relationships of man might be further cleared by the study of the iso-agglutinating factors in animals.

The differentiation of human and animal blood in the past has largely been attempted on the basis of the precipitin test. Such tests with human blood and the blood of anthropoid apes and monkeys have been made by Gruübaum (1902), Nuttall (1904), Chio (1905), Friedenthall (1900), Marshall (1901), Ulenhuth (1902), et al. In 1911 von Dungern and Hirszfeld made the first observation on anthropoids from the standpoint of agglutinins. They studied two sets of chimpanzee serum and one set of erythrocytes. The bloods corresponded to the human group *A*. They were unable, however, to group the cells of macaque monkeys with human serums.

Landsteiner and Miller (1925) examined the blood of 21 anthropoid apes, and 76 lower monkeys of 36 species. The erythrocytes were grouped by means of human serums or adsorbed immune rabbit serums. Since hetero-agglutinins for anthropoid corpuscles are present in human serums

the investigators had first to obviate the effects of these, which they did in some instances by absorbing the iso-agglutinins and then freeing them again by themselves. In other instances they used group-specific antiserums obtained by the immunization of rabbits with human group *A* and group *B* cells, respectively.

Of the 21 anthropoid apes studied, 18 possessed iso-agglutinogens in the red cells, which were found to be identical with those of human blood. Of the 14 chimpanzees studied, 11 belonged to group *A* and 3 to group *O*. Of

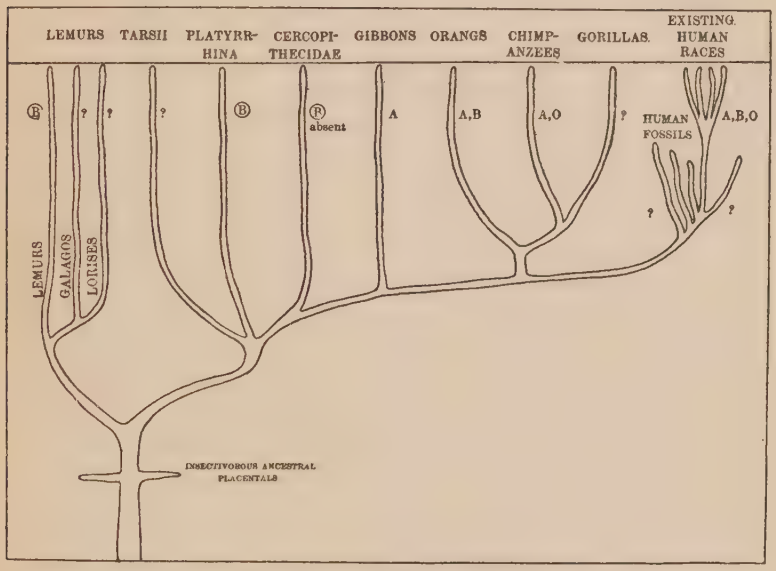

Fig. 25. The Blood Groups in Apes and Monkeys

Redrawn from Landsteiner and Miller 1925

the 6 orang-utans, 2 belonged to group *A*, 3 to group *B*, and 1 to group *AB*. The 1 gibbon studied belonged to group *A*. In the lower monkeys, only a factor similar to human iso-agglutinogen *B*, but not identical with it, was found. In figure 25 is given a schematic representation of the iso-agglutinins now known to be found in the erythrocytes of the primates. Further investigation will probably extend this list, as relatively small numbers have thus far been studied.

In the lower vertebrates no such definite results have been obtained. Hektoen (1907) made the first direct tests on animal blood. He studied rabbits, guinea-pigs, dogs, horses, and cattle, but was not able to find

any iso-agglutination, although from 10 to 20 individuals of each species were used. Von Dungern (1910) concluded from experiments by the absorption method that there were four groups in dogs. In 1911, Ottenberg and Friedman studied rabbits and steers, and found that the bloods of 32 rabbits fell into four groups, while the blood of 11 steers could be placed in three groups. To account for this, they assumed the presence in the bloods of rabbits of two iso-agglutinins and two agglutinogens; in the bloods of the steers they assumed the presence of one of each.

Ingebrigsten (1912) studied 40 cats. In 5 cases he found inter-agglutination, but most of his results were negative, and no satisfactory grouping could be made. He likewise failed to find iso-agglutinins in dogs. Ottenberg, Friedman, and Kalisky (1913) confirmed the lack of groups in dogs, finding only occasional iso-agglutination. In 1915 Ottenberg and Thalhimer likewise confirmed the lack of groups in cats. In 1913, Fishbein studied several species, including 60 swine, 60 cattle, 40 sheep, 25 rabbits, 10 dogs, and 20 frogs. He found only occasional iso-agglutination, and concluded that the phenomenon was much less pronounced in animals than in man, and permitted of no satisfactory grouping.

Weszeczky (1920) found no iso-agglutination in cattle, rabbits, guinea-pigs, and chickens. In dogs, horses, and swine he found occasional iso-agglutination, but was unable to make any groupings. Rhodenberg (1920) failed to find agglutination in 50 rats of various strains. Likewise MacDowell and Hubbard (1922) did not observe agglutination in 1180 combinations of serum and cells of diverse strains of mice.

Pannisset and Verge (1922) could not detect any evidence of fixed blood groups in either horses or cattle. Przesmycki (1923) distinguished three groups in horses similar to the iso-agglutination groups in man, but less regular. Landsteiner and van der Scheer (1924) found a similar grouping. Walsh (1924) found irregular iso-agglutination in horses, but could not demonstrate any grouping. Schwartz (1926) was also able to demonstrate the presence of iso-agglutinins in horse blood. Newodow (1928), in a study of the bloods of 1730 horses, reported the presence of four groups analogous to the human groups. Schermer (1929) reported similar results.

Zwetkow (1927) found several groups and sub-groups in dogs. Bialosuknia and Kaczkowski (1924) reported the occurrence of three groups in sheep. Karshner (1928, 1929) studied iso-agglutination in cattle and chickens. In cattle he found no iso-agglutination within the breed, but occasional reactions when different breeds were cross-agglutinated. The phenomenon was less common than in human bloods, but he was able to distinguish two groups, a large group, the cells of which are not agglutinated, but the serum

of which agglutinates the cells of a smaller group. The serum of the smaller group has no agglutinating capacity. In chickens similar results were found and three groups could be designated.

In 1924 I attempted to find blood groups in rabbits for the purpose of an experimental study of the inheritance of iso-agglutinating factors. The technic is here given in detail as indicative of one of the available methods for such studies. Practically every known variety of rabbit was used, the strains varying from the large Flemish Giant to the small Polish rabbit, and from stock which had been in American laboratories for generations to freshly imported animals.

The rabbits were bled from the marginal ear vein. The skin over the vein was shaved and rubbed with xylol to induce hyperemia. A hot cloth was applied at the base of the ear. The blood was drawn through a no. 22-gauge needle into a 10-cc. syringe. Three drops of blood were added to 10 cc. of sodium citrate solution (1 per cent sodium citrate in isotonic saline). This gave a 2 per cent suspension of blood without clotting. This suspension was centrifuged, and the supernatant fluid pipetted off. The original amount of solution was restored by adding isotonic saline.

The remaining portion of the blood taken from the rabbit was allowed to clot in a centrifuge tube. After an hour at room temperature, the clot was gently loosened. The tubes were allowed to stand overnight in the ice chest. The clot was then whirled down, and the serum transferred to a sterile vial. Sterile apparatus and rigid aseptic technic were used throughout the tests.

In this case the macroscopic method of reading was used. In the future I expect to use only the microscopic method, which I consider more accurate even though more time is needed. For the macroscopic method, 0.2 cc. of serum were added to 0.1 cc. of washed corpuscles in small vials. The vials were then placed in the incubator at 37°C. for an hour, after which the first reading was made. They were then placed in the ice chest, and several subsequent readings made at intervals of an hour, and again after the vials had stood over night in the ice chest. The microscopic method takes much less serum and corpuscles for each test, and in that way many more combinations are possible with limited amounts of serum. In this instance microscopic observations of hanging drops were made with 10 per cent of the observations to act as a check. In every instance the microscopic observation confirmed the macroscopic reading.

Each serum was tested against as many other corpuscles as possible. Its own corpuscles acted as a control. Serum from each strain was tested against corpuscles from each of the other strains. About two thousand

combinations of serum and corpuscles were made, but no evidence of iso-agglutinins could be detected.

With regard to the agglutination of the cells of one species by the serum of another, some interesting results have been obtained. It has of course long been known that in general there is agglutination between the bloods of different species of animals. Interesting exceptions to this rule of hetero-agglutination have been noted. Weszeczky (1920) found that the serum of the buffalo did not agglutinate the cells of cattle. MacDowell and Hubbard (1922) recorded that mouse cells were not agglutinated by rat serum. Walsh (1924) found that horse serum did not agglutinate ass cells, although in most cases ass serum agglutinated horse cells. Mule serum did not agglutinate either horse or ass cells, nor were mule cells agglutinated by horse or ass serum. Landsteiner and van der Scheer (1924) obtained similar results, but found that certain mule serums agglutinated certain horse bloods, while some mule cells were agglutinated by certain horse or ass serums.

Lack of agglutination has been assumed by the absence of reactions after transfusions in certain cases. Friedenthal (1900) found no ill effects in a cat after transfusion of ocelot blood into it. There were also no ill effects when transfusion was effected between rabbit and hare, or between rat and mouse.

Such results indicate taxonomic possibilities based on hetero-agglutinating reactions. Some apparently incompatible results on this basis have been obtained, however. It has been accepted as a general principle that human cells are constantly agglutinated by animal serums (Marx and Ehrnrooth 1904, Williams and Patterson 1910, Kolmer and Matsumoto 1920, Kolmer and Trist 1920). Karshner (1928, 1929) found that although chicken serum constantly agglutinated human red cells, human serum seldom agglutinated chicken cells. Group *A* serum especially failed to agglutinate chicken cells. Human serum likewise failed in about 4 per cent of the cases to agglutinate bovine cells. Perry and Rhodes (1929) showed that human serum constantly agglutinated sheep cells. A wide field is open in the study of taxonomic relationships based on iso- and heteroagglutination.

Iso-agglutination within a species of lower animals has not as yet been established beyond a doubt. Contradictory reports occur for practically every species. It may be safely stated that the phenomenon of iso-agglutination, if it does occur in animals, is certainly less common and probably less regular than in human beings. The clear establishment of definite blood groups in any laboratory animal would provide for the experimental study of blood group inheritance and behavior, a possibility to be hoped for and carefully investigated.

CHAPTER XIV

The Racial Distribution of the Blood Groups

"Die allgemeinem genetischen Vorbedingungen für eine rassenkundliche Verwendung sind bei den Blutgruppen mindestens ebensogut, z. T. sogar noch besser erfüllt, als bei anderen Merkmalen, die sich zur Rassenunterscheidung schon bewährt haben. . . . . Auch für die Blutbeschaffenheit, die sich in der Isohämagglutination ausdrükt, kann demnach von vornherein nicht deshalb ein besonderer rassenkundlicher Wert erwartet werden, weil es sich um Blut handelt."—Scheidt, *Rassenunterschiede des Blutes*, 1927.

One of the most striking and unexpected results of the discovery of "blood groups" within the human race has been the extensive application of blood group distribution data to anthropology. The idea that races carry distinguishing characters hidden in the blood has laid strong hold on the imagination of medical and other research workers; so much so that the idea has been slightly overdone. Many workers in the flush of the new field of operations have not realized that the blood groups are simply additional anthropological characters which must take their place along with other longer-known criteria in the study of racial relationships.

The groundwork for the present extensive research on the racial phase of the blood group problem was laid by L. and H. Hirszfeld on the Macedonian battlefront during the World War. As army physicians, they took advantage of the concentration of races in the war area, and grouped the bloods of 500 members of each of 16 different peoples. The results demonstrated that the percentages of the four groups vary in different peoples, and that this variation is roughly correlated with geographic distribution. Figure 26 shows the original results as reported by the Hirszfelds (1919).

The Hirszfelds' work was a powerful stimulus to further investigation. Blood group distributions on peoples from all parts of the world poured into the literature. Because of the nature of the blood group tests, much of the work has been done by physicians and reported in medical journals, although of recent years geneticists and anthropologists have been active in the work. Hirszfeld (1927) records that a reputable journal, after keeping the manuscript of the original work for nine months, returned it with the comment that the subject matter would not interest physicians. Yet today, ten years later, there are several journals devoted to blood grouping alone, while articles on the various phases of the subject, written largely by

medical men, pour almost daily into medical, genetical, and anthropological journals.

A summary of the Hirszfelds' original work shows that factor A decreases from west to east, geographically, while factor B increases. The 16 peoples studied in this investigation could be roughly divided into three types, based on the relative amounts of A and B. As a serologic criterion the Hirszfelds suggested a "biochemical index," to be arrived at by dividing the percentage of A in a given race by the percentage of B; that is, by dividing the percentage of groups $A + AB$ by the percentage of groups $B + AB$. On this

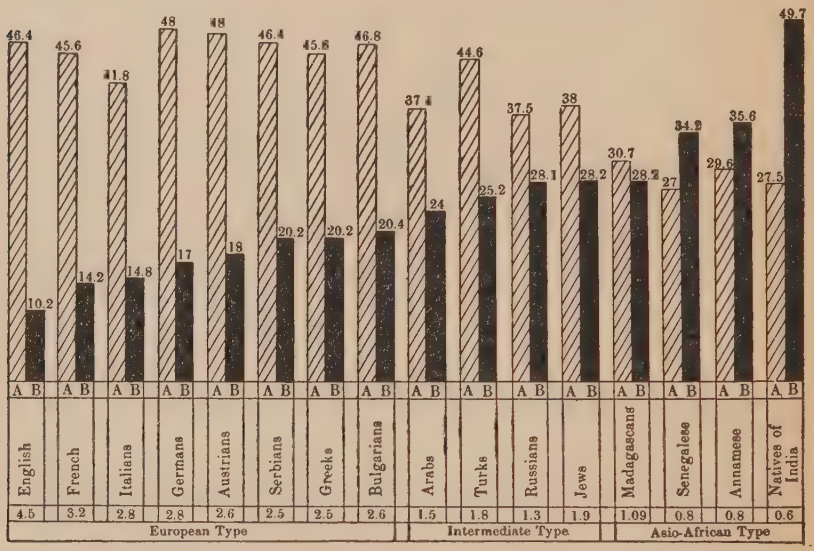

FIG. 26. THE ORIGINAL SEROLOGIC RACIAL ANALYSIS

Redrawn from Hirszfeld and Hirszfeld 1919

basis there evolved three types, a European type having an index of 2.5 or over (more than twice as much A as B); an Intermediate type having an index of 1.3 to 1.8; and an Asio-African type having an index of 1.0 or less. Later reports from many investigators have given indices of intermediate magnitudes, so that no sharp divisions exist at present on this basis. Moreover, as I have several times pointed out, the "biochemical index" is not mathematically adequate, and must be discarded. Some of the original reports of investigations gave the biochemical index of the race studied, with no other data. It can readily be seen that this is useless. Two races, each having an equal proportion of the agglutinogens A and B, and thus

having the same "intermediate index," might differ in that one had, for instance, 40 per cent A and 40 per cent B, with a small percentage of group *O*, while the other had only 3 per cent A and 3 per cent B, with a correspondingly large proportion of group *O*. The races would be obviously different, yet the biochemical index would be the same. In spite of this, however, the biochemical index is still reported in the publication of researches. As will be pointed out later, correlation charts involving the frequencies p, q, and r, are the only correct methods of interpreting the racial data.

Other indices have been subsequently suggested by various writers, so that there exist at present six different forms of the original biochemical index, none of which in my opinion is worth anything. The six indices which have been suggested are as follows:

(1) Biochemical race index (Hirszfeld and Hirszfeld) $= \frac{A + AB}{B + AB}$

(2) Serological group index (Leveringhaus) $= \frac{O + A}{O + B}$

(3) Biological race index (from Bernstein's formulae) $= \frac{p}{q}$

(4) Serological regression index (Wellisch) $= \frac{(A + AB)\ (O + B)}{(B + AB)\ (O + A)}$

(5) Serological race index (Lattes) $= \frac{\text{group A}}{\text{group B}}$

(6) New biochemical race index (Melkich) $= \frac{O + A}{B + AB}$

With the exception of index (6), the following general statements hold true:

When A = B, all indices = 1
When A < B, all indices < 1
When A > B, all indices > 1

When various peoples are arranged in order according to one of the indices, they are also in order generally in regard to the others.

The frequencies p, q, and r, however, are much more important than the percentages of the groups as such. These frequencies may be calculated for any race, as shown in chapter X. When this is done, we have accurate figures which may be applied to the study of racial relationships.

Ottenberg (1925), perceiving that the biochemical index was not entirely adequate, presented a classification of peoples based on the percentages of the groups. He recognized six types. It is possible, however, to make a more accurate classification based on the frequencies p, q, and r. I have

q \ p	0–5	6–10	11–15
0–5	American Indians (full-blooded)	American Indians Eskimos	
6–10		Filipinos	
11–15			South Africans Yoruba
16–20			Senegalese Sumatrans
21–25			
26–30			Natives of Indi Gypsies

FIG. 27. CORRELATION OF p AN

16–20	21–25	26–30	31–36
	Australians		
anders	Danes Greenlanders Argentinians	Americans English French Italians Maltans Germans Austrians Dutch Belgians Serbs Greeks Lapps	Norwegians Swedes Portugese
rican Ne- oes legascans nesians occans aicans ac	Arabs Turks Russians Slovaks Spanish Jews	Finns Roumanians Bulgarians Polish Jews	Armenians Lithuanians
ngans mese nese atran inese	South Chinese	South Chinese North Japanese Hungarians Poles	Middle Japanese South Japanese Formosans Roumanian Jews
h Koreans ko	Middle Koreans	Egyptians	South Korean Ukranians
h Chinese chus		Ainu	Ainu

MONG THE PEOPLES OF THE WORLD

made such a classification, (Snyder 1926, 1927), and the result is illustrated in figure 27.

Steng (1926) presented a graphical chart theoretically in three dimensions, but actually a plane due to the limitations of flat printing. Three axes, PP, QQ and RR, are used, corresponding to the frequencies of the hereditary factors, and the position of each people on these axes is plotted from the frequencies of the three factors in that people.

My correlation chart as shown in figure 27 is more easily understood. In this figure the frequencies p and q are grouped on a correlation table; the frequency r is ignored, as two races having similar frequencies of p and q would have similar frequencies of r, since $p + q + r = 1$. If desired, values of r could be added to this chart as a third dimension.

The various peoples on the chart fall more or less into natural groups. A certain empiric manner of grouping must be admitted, since p and q have been lumped into 5 per cent classes. Each square in this figure, however, contains nationalities or races which agree in their frequencies p, q, and r. It must be emphasized, however, as I have repeatedly done, that it is not implied that because two peoples occur in the same portion of the figure, they have the same racial history, but only that they contain similar amounts of A and B.

For convenience, the various peoples in figure 27 have been arbitrarily divided into types, set off by heavy lines. These types will be briefly discussed.

1. The European type. This is a group of peoples showing a high frequency of A, coupled with a low frequency of B. The amount of A is surpassed only by some peoples of the Hunan Type, a fact which brings up a problem to be discussed later in this chapter. The amount of B is the lowest of any race with the exception of the American and Australian aborigines. It seems reasonable to assume that factor A originated in west Europe and spread eastward by successive waves of migration. The frequency of A decreases from north-west to south-east in Europe, and continues to decrease to the east, reaching its lowest point in India. Certain exceptions to this rule occur, notably Japanese, Koreans, and Australian aborigines. These will be discussed later.

2. The Intermediate type. This group of peoples lies generally intermediate in geographic position between Europe, with its high frequency of A, and Asia, with its high frequency of B, and shows correspondingly an intermediate condition of the frequencies of A and B. The individual studies on these peoples are in many cases of especial interest, and should be consulted in the original. They may be found by looking up the references

in the bibliography according to the authors as given in table 21 at the end of this chapter. For example, Popoviciu (1924) studied the blood groups of the Roumanians in the mountains and in the valleys. The frequency of A falls in the valleys, while the frequency of B rises. Popoviciu attributed this to the fact that the valleys have been open and accessible to admixture from eastern peoples, while in the inaccessible mountains the race is in more nearly its early condition. Again, in Syria, where the races divide along religious lines, Parr finds a striking difference between the blood group distribution of Christian Arabs and that of Moslem Arabs, although for several centuries they have been living together along the coast, speaking the same Arabic, wearing largely the same clothes, and looking alike physically.

3. The Hunan type. This type originally derived its name from the fact that South Chinese of the province of Hunan were included in it. Later researches on Chinese have shown varying proportions, and North and South Chinese may prove to be closer in blood group proportions than was originally thought. The Hunan type contains those peoples with an exceptionally high percentage of A, and a moderately low percentage of B. It includes Japanese, Koreans from that part of Korea nearest Japan, and South Chinese; in addition it includes Hungarians, Poles, Ukranians, and Egyptians, suggesting the possibility of Mongol relationships in the ancestry of these peoples.

It is evident that southern Korea and adjacent southern Japan provided a center for the spread of agglutinogen A in addition to the center already seen to be located in northern Europe. The factor A may have originated in this additional center by an independent mutation, or it may have been brought there by races of the European type early in the history of the differentiation of the surrounding peoples. It is believed that early in the phylogeny of the Asiatic Mongols there was differentiated a race which developed into the Koreo-Japanese peoples. This race was at its differentiation strongly infused with Caucasian elements. Korea was the original home of the group. This belief is supported by the available blood group data. The high frequency of A drops rapidly as we radiate from this center. Thus, the value of p drops from 32.5 in south Korea to 19.4 in north Korea, and 19.5 in Manchuria; it drops from 34.5 in south Japan to 27.4 in north Japan. The value of q, however, remains rather constant in these regions.

4. The Indomanchurian type. This type contains those peoples who show a high frequency of B and a rather low frequency of A. The peak of the B frequency is reached in India and Asia, diminishing to the east, and reaching its lowest ebb in Europe. This may be accepted as evidence that

mutation B took place somewhere in Asia, after the American Indian branch had become separated (see Pacific-American type below) and spread westward from there.

It will be noted that North Chinese, North Koreans, and Manchus are included here, in contrast to South Chinese, South Koreans, and Japanese who are included in the Hunan type. The Gypsies, which are included in the Indomanchurian type, were originally from India, and agree in blood group proportions with the present natives of India, several centuries after the separation of the two peoples.

The Ainu are included in this group, although both agglutinogens are especially well developed in these people, and they practically deserve a type to themselves. The Ainu are proving a puzzle from the standpoint of the blood groups, just as they have long been an anthropological puzzle.

5. The Africo-Malaysian type. This is a fairly homogenous type, including all of the African and Malay peoples studied. Both agglutinogens are moderately well developed, and this is as true of the blacks of the Pacific Islands as of those of Continental Africa.

6. The Pacific-American type. This type includes American Indians of North, Central, and South America; Eskimos; and Filipinos. The type is characterized by a large frequency of r, and correspondingly small frequencies of p and q. My studies on American Indians have shown that full-blooded Indians are probably all of group *O*, the occurrence of the other groups being due to white admixture. The small amounts of A and B present in American Indians are in the relative proportions in which they are found in white Americans. Similarly, the small amounts of A and B found in Filipinos are in the relative proportions occurring in the surrounding Malayan and Ethiopian peoples, suggesting that full-blooded Filipinos would also be of group *O*. It would thus appear that these races were isolated from the Eurasic continent before either mutation took place.

7. The Australian type. The Australian aborigines show a high frequency of A, comparable with the peoples of the European type. The extremely low frequency of B makes it inadvisable to consider them with this type, however. Unlike the other islands of the Pacific, in which both agglutinogens are well developed, Australia harbors a race showing every indication of early isolation. The occurrence of group *O* reaches well over 50 per cent. Although Australia was open to migration from Malasia and India in Pliocene times, the Australians could not have received their present proportions of the blood groups from the black or brown races of either country as we know them today. The frequencies of the Australians are such as to suggest early isolation, followed by invasion by some race not

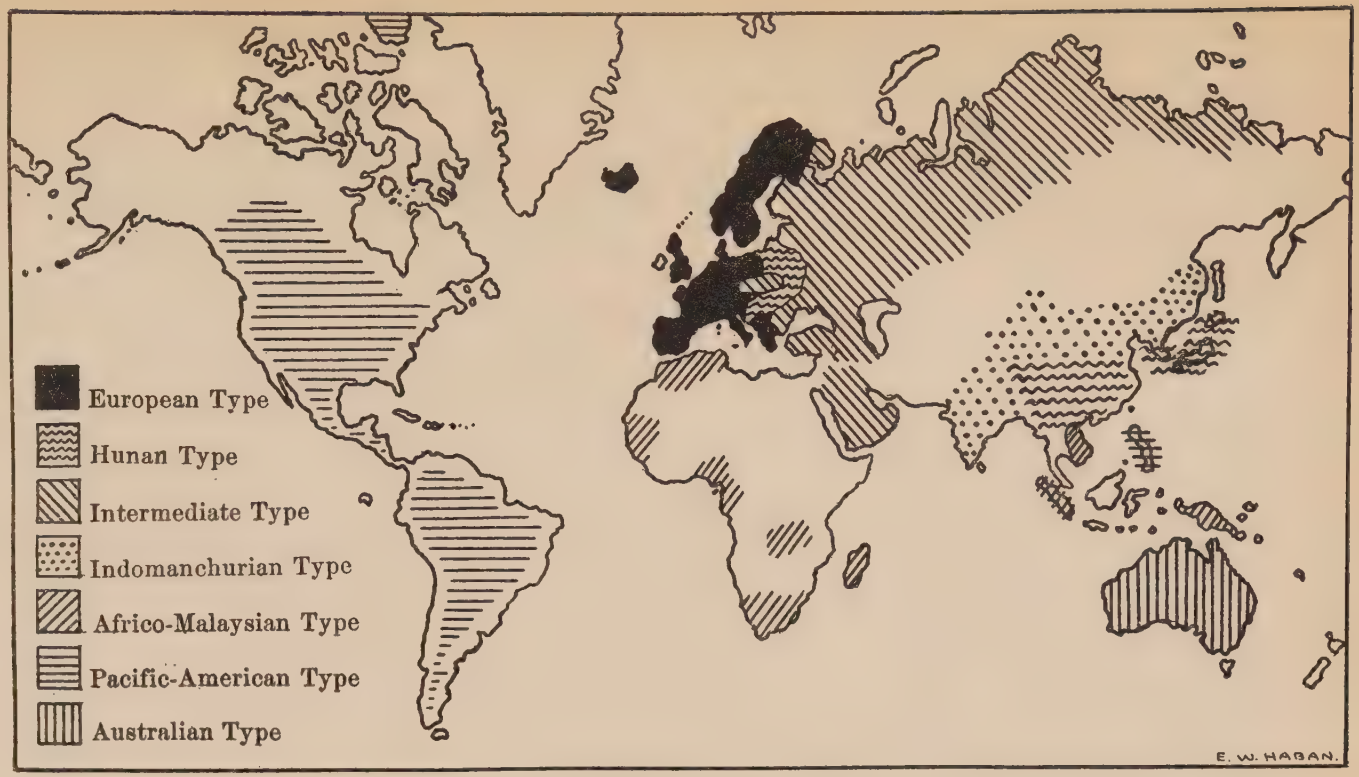

FIG. 28. THE WORLD DISTRIBUTION OF THE SEROLOGIC RACIAL TYPES

unlike the Caucasian races of today. In this connection a knowledge of the proportions of the blood groups of the Dravidians would be of extreme interest, and it may be predicted that when studied they will show a high frequency of A, unlike that of the surrounding Indian peoples. It is to be regretted that it is too late to learn the blood group distribution of the Tasmanians.

The world distribution of the seven types is shown in figure 28, and the detailed racial data is given in table 21 at the end of this chapter.

It must be remembered that these types are purely arbitrary, that they are subject to constant revision, and that they merely serve as a working basis for the serological classification of races. The grouping of peoples in figure 27 corresponds so closely to that based on other anthropological data, however, that the conclusion seems warranted that the blood groups may profitably serve as additional criteria in determining racial relationships. Their value, however, must not be over-weighted. Anthropological problems will not be solved from blood group data alone.

The blood group factors do not provide a more potent source of race-classification data than any other hereditary factors, with this exception: there is no possibility of a conscious selection in the case of the blood groups. Any selective value which the groups may have could be due only to a secondary relation with some other factor which did possess a selective value of its own. Nothing of this nature has as yet been demonstrated (see chapter XII). It must not be thought, however, that because the groups are hidden in the blood, they possess some mysterious power of providing a basis for racial classification. They must merely take their place as available criteria along with pigmentation, hair form, cephalic index, and the rest.

The heredity, stability, and non-selective value of the blood groups having been established, two corollary expectations may be demonstrated. Thus it is to be expected that the proportions of the groups would remain constant from generation to generation in any race in the absence of crossing; also that when crossing did occur, a definite change in the proportions of the groups would be brought about.

The first of these expected results has been demonstrated for many peoples for varying lengths of time. The most striking of these was the demonstration by Verzar and Weszeczky (1922) in the case of gypsies. They found that gypsies in Hungary, being originally from India but having lived to themselves in Hungary for several hundred years, showed the same distribution of the groups as the natives of India studied by Hirszfeld and Hirszfeld, quite different from those of the Hungarians surrounding them. The actual figures are given in table 19.

The second of these expectations, that racial crossing should modify the proportions of the groups, was demonstrated by me in 1926. A study of American Indians revealed the fact that they were mostly all of group *O*, and that the small amounts of groups *A* and *B* occurred largely in the mixed-blooded Indians. It was possible to work out a graded series from full-blooded Indians to whites, showing a constant increase in the agglutinogens. These results are shown in table 20.

Similarly, the Jamaicans, who have undergone considerable crossing with the whites, show blood group frequencies definitely nearer those of the

TABLE 19

ILLUSTRATING THE CONSTANT PERCENTAGES OF THE GROUPS IN THE ABSENCE OF CROSSING

RACE	NUMBER STUDIED	PER CENT IN GROUP			
		O	*A*	*B*	*AB*
Natives of India	1000	31.3	19.0	41.2	8.5
Gypsies (in Hungary)	385	34.2	21.1	38.9	8.5
Hungarians	1500	31.0	38.0	18.8	12.2

TABLE 20

GRADED SERIES SHOWING THE EFFECT OF CROSSING ON THE PROPORTIONS OF THE GROUPS

RACE	NUMBER STUDIED	PER CENT IN GROUP			
		O	*A*	*B*	*AB*
Indians said to be pure	453	91.3	7.7	1.0	0.0
All Indians (mixed and pure)	1134	79.1	16.4	3.4	0.9
Indians known to be mixed	409	64.8	25.6	7.1	2.4
Americans (white)	1000	45.0	42.0	10.0	3.0

whites than do the Yoruba, an unmixed tribe living in southern Nigeria, the origin of the ancestors of the Jamaicans.

From the results as presented above, we may reach several tentative conclusions, which I offer in the form of "laws" of serologic race-classification.

1. Any people being studied from the standpoint of blood groups may be expected to show blood group frequencies similar to those of other peoples known to be related to it.

2. If any people shows blood group frequencies different from those to be expected based on the frequencies of other peoples known to be related to it, the conclusion may be drawn that the former has undergone racial crossing of some sort which the latter have not undergone.

TABLE 21

PERCENTAGES OF THE FOUR BLOOD GROUPS AMONG DIFFERENT PEOPLES, WITH THE FREQUENCY OF EACH FACTOR CONCERNED

RACE	INVESTIGATOR	NUMBER	PER CENT IN GROUP						
			O	*A*	*B*	*AB*	p		r
European Type									
Americans	Hektoen 1907	—	46.4	43.4	7.2	3.0	26.8	5.2	68.1
	Moss 1910	1,600	43.0	40.0	7.0	10.0	29.3	8.9	65.6
	Buchanan and Higley 1921	1,536	46.9	40.8	8.5	3.6	25.6	6.4	68.4
	Culpepper 1921	5,000	41.0	38.0	18.0	3.0	23.2	11.2	64.0
	Ottenberg 1921	286	44.0	42.0	12.0	2.0	25.2	7.3	66.3
	Snyder, to date	20,000	45.0	41.0	10.0	4.0	25.9	7.3	67.0
English	Hirszfeld and Hirszfeld 1919	500	46.4	43.4	7.2	3.0	26.8	5.2	68.1
Scotch	Alexander 1921	225	43.6	33.9	16.8	5.7	22.3	12.0	66.0
French	Hirszfeld and Hirszfeld 1919	500	43.2	42.6	11.2	3.0	26.2	7.4	65.7
Belgians	Staquet 1925	1,072	47.9	41.8	7.1	3.2	25.8	5.3	69.2
Italians	Hirszfeld and Hirszfeld 1919	500	47.2	38.0	11.0	3.8	23.7	7.7	68.7
	Cavalieri 1922	139	35.9	51.0	8.6	4.1	33.3	6.8	59.9
	Mino 1924	1,391	35.9	51.1	8.6	4.2	33.3	6.9	59.9
Maltans	Snyder 1926	150	46.0	44.0	6.0	4.0	27.9	5.2	67.8
Germans:									
Heidelberg	Hirszfeld and Hirszfeld 1919	500	40.0	43.0	12.0	5.0	27.9	8.9	63.2
In Hungary	Verzar and Weszeczky 1922	476	40.8	43.5	12.6	3.1	26.9	8.2	63.8
In Switzerland	Plüss 1924	543	42.6	43.1	8.8	5.5	28.4	7.5	65.2
Berlin	Schiff and Ziegler 1924	750	37.8	39.4	16.4	6.4	26.4	12.1	61.5
Schleswig-Holstein	Schütz and Wöhlisch 1924	1,679	42.7	42.7	11.7	2.9	26.3	7.6	65.3
Leipsig	Sucker 1924	1,000	34.5	41.5	16.5	7.5	28.6	12.8	58.7
Keil	Steffan 1924	500	39.8	42.8	14.0	3.4	26.7	9.2	63.0
Schleswig-Holstein	Gundel 1926	8,662	37.3	43.7	13.4	5.7	28.8	10.0	61.1

German Jews	Schiff and Ziegler 1924	230	42.1	41.1	11.9	4.9	26.6	8.8	64.9
Austrians	Hirszfeld and Hirszfeld 1919	500	42.0	40.0	10.0	8.0	27.9	9.5	64.8
	Hoche and Moritsch 1926	1,000	33.1	39.9	20.1	6.9	27.2	14.6	57.4
Dutch	Snyder 1926	200	42.0	44.0	9.0	5.0	28.6	7.3	64.8
Norwegians	Jervell 1923	436	35.6	49.8	10.3	4.3	32.3	7.6	59.7
Swedes	Hesser 1924	533	36.9	46.9	9.7	6.4	31.8	8.5	60.7
	Lindberger 1925	500	33.5	51.0	10.0	5.5	34.1	8.1	57.8
	Rietz 1927	250	50.0	41.0	7.0	2.0	24.5	4.6	70.7
Danes	Johannsen 1921	150	47.3	36.7	12.0	4.0	23.0	8.3	68.8
	Johannsen 1925	512	43.0	42.0	12.0	3.0	25.9	7.9	65.5
Lapps	Rietz 1927	199	50.8	42.2	3.0	4.0	26.7	3.6	71.3
Icelanders	Jonsson 1923	800	55.6	32.1	9.6	2.6	19.2	6.4	74.6
Greenlanders	Bay-Schmith 1927	230	53.5	35.6	7.4	3.5	22.0	5.6	73.1
Portugese	Steffan and Wöhlisch 1928	459	38.4	52.5	6.1	3.0	33.3	4.7	61.9
Serbians	Hirszfeld and Hirszfeld 1919	500	38.0	41.8	15.6	4.6	26.8	10.7	61.8
Greeks	Hirszfeld and Hirszfeld 1919	500	38.2	41.6	16.2	4.0	26.2	10.7	61.8
	Kumaris 1928	716	43.8	31.9	12.7	4.4	24.9	13.0	66.1
Argentinians	Mazza and Franke 1928	654	49.0	39.0	9.5	2.5	23.5	6.2	70.0
Intermediate Type									
Finns	Streng and Ryti 1927	5,134	32.8	43.5	17.0	6.7	29.4	12.6	57.2
	Sievers 1927	478	32.0	43.7	19.1	5.2	28.5	13.0	56.6
Arabs	Hirszfeld and Hirszfeld 1919	500	43.6	32.4	19.0	5.0	20.9	12.9	66.0
Moslem Arabs	Parr 1929	795	33.8	36.8	18.5	10.8	27.7	15.9	58.1
Turks	Hirszfeld and Hirszfeld 1919	500	36.8	38.0	18.6	6.6	25.6	13.6	60.7
Russians	Hirszfeld and Hirszfeld 1919	1,000	40.7	31.2	21.8	6.3	21.0	15.2	63.8
	Avdeieva and Grizevicz 1925	2,200	32.0	38.5	23.0	6.5	25.9	16.1	56.6
	Feldmann and Elmanowitsch 1925	815	29.2	35.3	19.2	16.3	30.4	19.7	54.0
	Grusdieff 1926	510	32.0	34.0	26.0	8.0	25.2	18.8	56.6
	Eisenberg 1928	823	33.0	36.8	22.1	0.8	25.8	16.5	57.4

TABLE 21—*Continued*

RACE		INVESTIGATOR	NUMBER	PER CENT IN GROUP						
				O	*A*	*B*	*AB*	p	q	r
Intermediate Type										
Slovaks		Manuila 1924	461	44.7	31.3	15.8	8.2	22.3	12.9	66.8
		Kossovitch 1925	212	39.8	40.0	12.4	7.8	28.2	11.1	62.6
Spanish Jews		Hirszfeld and Hirszfeld 1919	500	38.8	33.0	23.2	5.0	21.3	15.3	62.3
Roumanians		Manuila 1924	1,521	33.7	43.3	15.6	7.4	29.8	12.3	58.2
		Weszeczky 1920	81	32.1	34.5	16.1	17.3	30.6	18.4	56.6
	Mountains	Popoviciu 1924	2,372	36.5	40.9	14.5	7.9	28.6	12.1	60.4
	Valleys	Popoviciu 1924	1,278	33.5	41.2	19.0	6.3	27.5	13.6	57.8
		Mironescu and Stefanoy 1926	152	23.7	46.7	19.1	10.5	34.6	16.1	48.7
Bulgarians		Hirszfeld and Hirszfeld 1919	500	39.0	40.6	14.2	6.2	27.1	10.8	62.4
	In Roumania	Manuila 1924	372	31.5	45.4	14.8	8.3	32.0	12.4	56.1
		Seisow and Zontschew 1929	1,000	30.6	44.8	16.5	8.1	31.3	13.1	55.3
Polish Jews		Halber and Mydlarski 1925	818	33.1	41.5	17.4	8.0	28.9	13.6	57.5
Armenians		Altounyan 1927	653	27.0	53.0	14.0	6.0	36.0	10.6	51.9
		Kossovitch 1927	380	36.3	40.3	16.6	6.8	27.3	12.5	60.0
		Parr 1929	1,536	28.3	46.7	12.6	12.4	36.1	13.4	53.2
Lithuanians		Hilgers et al 1928	500	—	—	—	—	30.4	10.6	57.6
Hunan Type										
South Chinese:										
Hunan		Chi-Pan 1924	1,296	31.8	38.8	19.4	9.8	28.5	16.0	56.3
Setshuan		Liang 1924	—	44.8	28.9	23.7	2.6	17.3	14.2	66.9
Tschekiang		Liang 1924	—	37.0	29.8	22.5	10.7	22.9	18.3	60.8
Kuangtung		Liang 1924	—	40.0	31.4	23.8	4.8	20.1	15.6	63.2

North Japanese:									
Sendai	Matsubara 1920	151	32.5	37.0	19.2	11.3	28.1	16.6	57.0
Sendai	Ninomiya 1925	642	29.4	39.3	21.5	9.8	28.7	17.2	54.2
Sendai	Mitomo (from Ninomiya 1925)	468	29.1	39.7	21.6	9.6	28.8	17.1	53.9
Niigata	Miyaji (from Sievers 1927)	1,786	30.2	37.9	22.5	9.5	27.4	17.5	55.0
Middle Japanese:									
Nagano	Hara and Kobayashi 1916	353	24.4	40.5	16.0	20.0	36.8	19.7	49.0
Tokyo	Nakajima (from Ninomiya 1925)	501	31.5	38.5	22.4	8.0	26.6	16.4	56.1
Tokyo	Shirai (from Ninomiya 1925)	317	30.5	37.5	21.5	10.4	27.9	17.6	55.2
Kyoto	Nakijima (from Ninomiya 1925)	509	28.7	41.7	20.2	9.4	30.1	16.1	53.5
South Japanese (Fukuoka)	Torii 1922	87	23.0	46.0	20.0	11.0	34.5	17.0	47.9
Japanese (in Korea)	Kirihara 1924	502	29.4	42.2	20.6	7.8	29.3	15.4	54.2
South Koreans (Zennan)	Kirihara 1924	171	19.9	41.5	25.7	12.9	32.5	21.6	44.6
Hungarians	Weszeczky 1920	457	26.3	38.1	18.8	16.8	32.9	19.8	51.2
Hungarians	Verzar and Weszeczky 1922	1,500	31.0	38.0	18.8	12.2	29.4	17.0	55.6
Hungarians	von Jeney 1923	1,172	22.3	31.6	27.4	18.7	29.6	26.6	47.2
Hungarians, In Roumania	Manuila 1924	688	27.8	40.8	20.2	11.2	30.7	17.2	52.7
Hungarians, In Roumania	Weitzner 1926	1,000	35.7	43.3	15.7	5.3	28.3	11.4	59.8
Poles	Halber and Mydlarski 1925	11,488	32.5	37.6	20.9	9.0	26.9	16.3	57.0
Poles	Amsel and Halber 1925	2,928	33.5	38.2	19.9	8.3	26.9	15.3	57.9
Poles	Wilczkowski 1927	365	31.7	38.9	19.7	9.8	28.3	16.0	56.4
Ukranians	Manuila 1924	400	18.0	39.2	22.5	20.3	36.4	24.4	42.4
Roumanian Jews	Manuila 1924	211	26.1	38.8	19.8	15.3	32.4	19.5	51.0
Egyptians	Wagner 1926	417	24.0	32.0	30.0	14.0	26.5	25.1	49.0
	Indomanchurian Type								
North Chinese:									
Pekin	Liu and Wang 1920	1,000	30.7	25.1	34.2	10.0	19.5	26.0	55.4
Shantung	Liang 1924	—	21.1	31.6	36.8	10.5	24.0	27.5	45.9
Nganhai	Liang 1924	—	47.9	21.7	21.7	8.7	16.6	16.6	69.1
Kiangsu	Liang 1924	—	39.0	29.7	26.4	4.9	19.2	17.2	62.4

TABLE 21—*Concluded*

RACE	INVESTIGATOR	NUMBER	PER CENT IN GROUP						
			O	*A*	*B*	*AB*	p	q	r
Indomanhurian Type									
Chinese	Kilgore and Liu 1918	100	28.0	36.0	25.0	11.0	27.2	20.0	52.9
Chinese (In United States)	Coca and Diebert 1923	111	29.0	32.0	29.9	10.0	23.8	21.9	53.8
North Koreans (Heihoku)	Kirihara 1924	354	30.5	27.4	34.5	7.6	19.4	23.9	55.2
Middle Koreans:									
Keiki	Kirihara 1924	311	27.3	32.8	32.8	7.1	22.5	22.5	55.2
Chuhoku	Kirihara 1924	112	17.9	36.6	33.7	12.5	28.2	26.2	42.3
Seoul	Fukamachi 1923	179	24.5	35.7	25.1	14.5	29.5	22.3	49.6
Phyengyang	Fukamachi 1923	184	31.5	29.9	27.7	10.8	23.1	21.6	56.1
Manchus	Fukamachi 1923	199	26.6	26.6	38.2	8.5	19.5	27.0	51.5
Manchus	Kirihara and Haku 1924	236	30.9	25.9	33.9	9.3	19.5	24.6	55.6
Indians (natives of India)	Hirszfeld and Hirszfeld 1919	1,000	31.3	19.0	41.2	8.5	14.9	29.1	55.9
Gypsies	Verzar and Weszeczky 1922	385	34.2	21.1	38.9	5.8	14.5	25.6	58.4
Gypsies	Hesch 1929	102	26.5	27.4	37.3	8.8	20.1	26.6	51.5
Ainu:									
Hokkaido	Ninomiya 1925	205	19.0	32.7	34.5	13.7	26.9	28.1	43.5
Karafuto	Kishi 1926	205	37.1	24.4	32.7	5.8	16.5	21.6	60.9
Hokkaido	Grove 1926	304	15.8	31.3	30.9	22.0	31.7	31.4	39.7
Orokko	Kishi 1926	89	30.3	24.7	37.1	7.8	17.9	25.8	55.0
Africo-Malaysian Type									
Senegalese	Hirszfeld and Hirszfeld 1919	500	43.2	22.6	29.2	5.0	14.9	18.9	65.7
South Africans	Pirie 1921	250	52.0	27.2	19.2	1.6	15.6	11.0	72.1
Madegascans	Hirszfeld and Hirszfeld 1919	400	45.5	26.2	23.7	4.5	16.8	15.4	67.5
Jamaicans	Snyder (unpublished data)	144	46.5	29.1	22.2	2.1	17.2	13.1	68.2

Moroccans		Snyder (unpublished data)	466	53.6	23.1	20.8	2.3	13.8	12.5	73.2
Katangans		Bruynoghe and Walravens 1926	500	45.6	22.2	24.2	8.0	16.5	17.7	67.5
Yoruba		Muller 1927	325	52.3	21.5	23.0	3.2	13.3	14.1	72.3
American Negroes		Lewis and Henderson 1922	270	49.0	26.9	18.4	5.5	18.0	13.0	70.0
		Snyder 1926.	500	47.0	28.0	20.0	5.0	18.2	13.4	68.5
Melanesians (New Guinea)		Heydon and Murphy 1924	753	53.7	26.8	16.3	3.2	16.4	10.3	73.2
Annamese		Hirszfeld and Hirszfeld 1919	500	42.0	22.4	28.4	7.2	16.1	19.8	64.8
Javanese		Bais and Verhoef 1924	1,346	39.9	25.7	29.0	5.4	71.0	19.1	63.1
Sumatrans		Bais and Verhoef 1924	546	43.7	23.0	29.0	4.3	14.8	18.4	66.1
Sumatran Chinese		Bais and Verhoef 1924	592	40.2	25.0	27.6	7.2	17.7	19.3	63.4
Gilyac		Kishi 1926	62	50.0	27.4	14.5	8.0	19.7	12.1	70.7
Pacific-American Type										
North American Indians	Full-blooded	Coca and Diebert 1923	862	77.7	20.2	2.1	0.0	10.7	1.1	88.0
		Nigg 1926	316	70.9	27.2	1.6	0.3	14.9	1.0	84.2
		Nigg 1926	457	72.7	26.9	0.2	0.2	14.6	0.2	85.2
		Snyder 1926	1,104	79.1	16.4	3.4	0.9	9.2	2.3	88.9
		Snyder 1926	453	91.3	7.7	1.0	0.0	4.0	0.6	95.5
		Landsteiner and Levine 1929	205	76.1	23.9	0.0	0.0	12.8	0.0	87.2
		Gates 1929	71	84.5	15.5	0.0	0.0	8.1	0.0	91.9
Maya Indians		Moss and Kennedy 1929	738	76.6	16.7	5.4	1.4	9.5	3.4	87.5
South American Indians		Mazza and Franke 1928	94	82.9	12.8	4.3	0.0	6.7	2.2	91.0
Eskimos		Heinbecker and Pauli 1928	124	80.7	12.9	2.4	4.0	8.8	3.3	89.8
Filipinos		Cabrera and Wade 1921	204	64.7	14.7	19.6	1.0	8.2	10.9	80.4
Australian Type										
Australian aborigines	Full-blooded	Tebbutt and McConnel 1922	204	57.0	38.5	3.0	1.5	22.6	2.3	75.5
	Full-blooded	Tebbutt 1923	191	54.9	38.2	5.7	1.0	21.9	4.4	74.3
	Full-blooded	Cleland 1926	101	45.5	54.5	0.0	0.0	32.6	0.0	67.4
	Full-blooded	Lee 1926	377	60.3	31.7	6.4	1.6	18.4	4.2	77.6
	Full-blooded	Cleland 1927	57	52.4	47.6	0.0	0.0	27.7	0.0	72.3

3. If any people shows blood group frequencies similar to a group of peoples not known to be related to it, the conclusion may be drawn that the former traces back to the latter somewhere in its ancestry, or else that the former has undergone crossing with the latter group or some similar people.

4. If any people lacks one or both of the blood group mutations, as evidenced by an extremely low value of p or q, it may be assumed that that people became isolated from the rest of mankind before the respective mutations took place, or before they spread very far.

Based on the fact that full-blooded American Indians and Filipinos seemed to be entirely of group *O*, and that factors A and B reach their greatest frequencies in Europe and Asia, respectively, it has been assumed by most writers that the human race was originally all of group *O*, the factors A and B having arisen separately by later mutation. This is further borne out by the fact that all the peoples showing more than 50 per cent group *O* are island folk or otherwise isolated. Recent studies on Eskimos further confirm this hypothesis.

Migration and intermingling probably constitute the agencies for the spread of the factors. Certain races such as the American Indians and Filipinos and probably the Australian aborigines must on this basis have been isolated from the rest of mankind before mutations A and B took place, or before there was any wide distribution of these factors. The low frequencies of these factors in the American Indians and Filipinos seem definitely due to intermingling. The frequency of A in the Australian aborigines suggests a definite invasion.

Continuing this hypothesis, mutation A probably occurred in Europe, and mutation B in India or the Orient, from which they were later carried and spread by migration. Factor A, while decreasing from Europe eastward, never becomes rare, even in the most eastern peoples. Factor B, on the other hand, becomes quite rare in western peoples, q reaching as low as 3 to 5 per cent. Several explanations for this may be conceived. Factor A may be older than factor B. Mutation A may have taken place originally in more individuals than did mutation B. Or the eastward migration of Caucasian peoples may have been more extensive or the intermingling more thorough than was the case with the westward migrations of Mongols. As a final assumption, only to be accepted after all other possibilities have been exhausted, an independent Asiatic mutation of A may be conceived. Migration maps and charts based on the various biochemical indices have been constructed by Steffan (1925–1929) and others.

Objections to this hypothesis have been raised on the ground that the

agglutinogens A and B have been shown to occur in the anthropoid apes. It does not seem necessary to imply, however, that these mutations were present in the direct ancestors of man, but rather that they arose independently in the anthropoids and man.

The validity of blood group distribution in any race as of anthropological significance has been questioned by various workers (Schütz and Wöhlish 1924, Lanner 1925, Grove 1926). These objections, based on the observations that the range of variation within a people may be as great as the variations between peoples, do not invalidate the anthropological importance of the groups. In the case of the Germans, the numbers studied in the various restricted localities are too small to involve statistically significant differences. In the case of the Ainu the various islands investigated are isolated, and the resulting inbreeding of small original stocks may well have carried on originally non-significant differences.

We may conclude, then, that the blood groups, phenotypic manifestations of definite unit Mendelian factors, take their place along with other hereditary characters, as aids in racial classification. Larger numbers of many races must be studied before we are justified in accepting more than tentatively any far-reaching conclusions, and the greatest need at present is the study of real races rather than nationalities or political groups. The blood groups seem destined to an important place in anthropology, however, just as they have reached outstanding positions in other branches of human biology.

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